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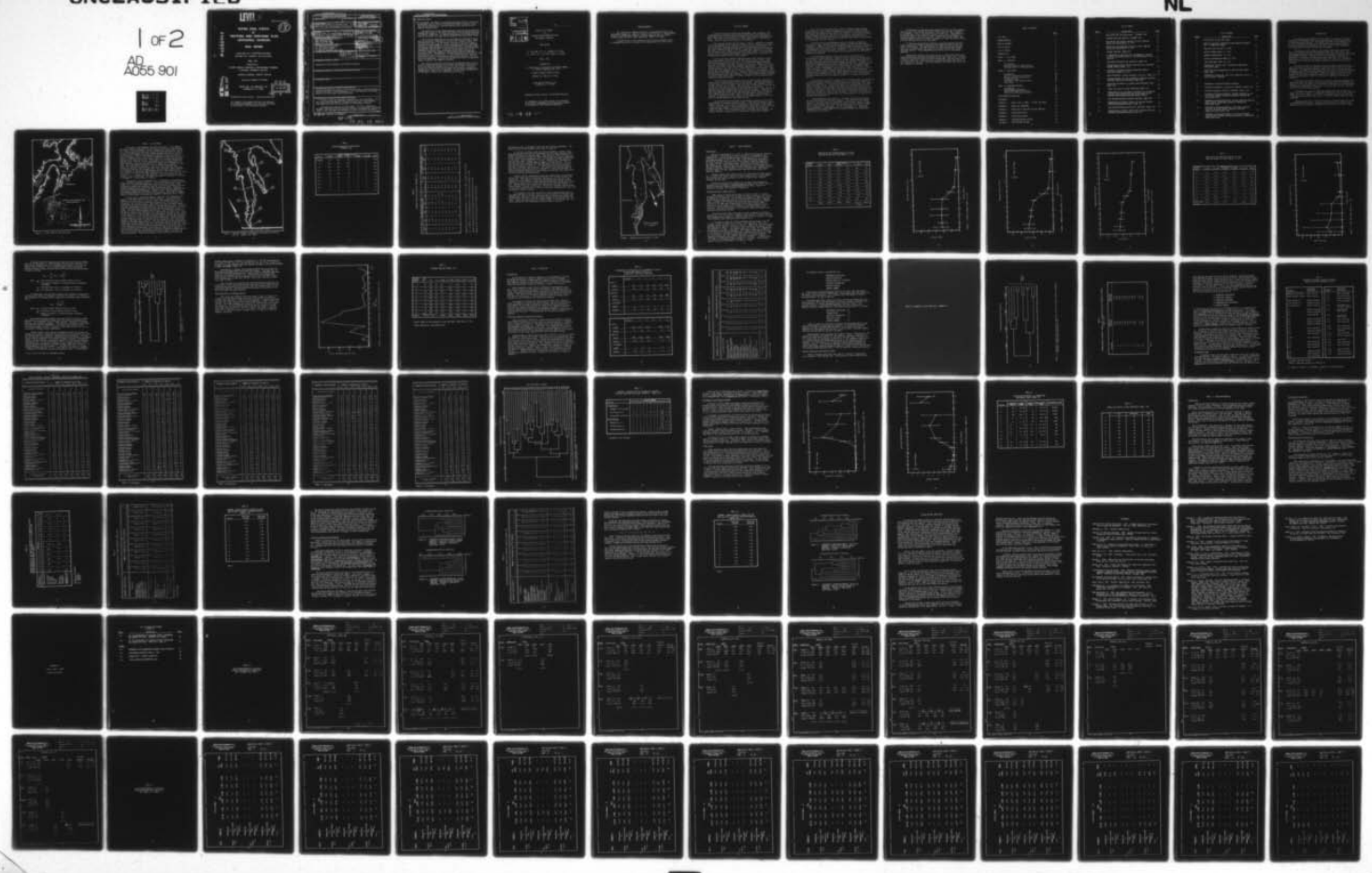

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WINTER FIELD SURVEYS AT VOLUNTEER ARMY AMMUNITION PLANT CHATTANOOGA, TENNESSEE

FINAL REPORT

J.H.SULLIVAN, JR., H.D.PUTNAM, M.A.KEIRN,
B.C. PRUITT, JR., D.R.SWIFT and J.T.Mc CLAVE

APRIL, 1978

Supported by

U.S.ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
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J.GARETH PEARSON, PROJECT OFFICER

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A winter survey was conducted in Waconda Bay, Lake Chickamauga. The Bay is the receiving water for TNT-manufacturing wastes from Volunteer Army Ammunition Plant located northwest of Chattanooga, Tennessee.

Field work was carried out in two phases. The first, completed in December, determined flow and dispersion patterns in Waconda Bay, munitions levels in the lake water and sediments and other limnological parameters cont.

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of the system. The Phase I survey determined that munitions levels were sufficiently high to justify an intensive biological study at a later date and provided data for locating sampling sites covering the full range of concentrations.

The second part of the investigation was completed during March and examined the effects of TNT wastes from VAAP on the periphyton and macroinvertebrate communities. A rigorous supplementary study determined the statistical variability occurring in periphyton colonizing glass slides.

Utilizing the population density and community structure of these selected biological compartments, estimates were made of effect levels from munitions waste. In the natural substrate no effects could be discerned in either number of species, population density, or community structure for both periphyton or macroinvertebrates at locations 3,000 feet downbay from the point of waste entry where total TNT concentration averaged less than 25 µg/l. During the 30 day exposure period for artificial substrates, samples of VAAP effluent were collected and analyzed for TNT. Concentration of 5 munitions compounds averaged 575 µg/l and ranged as high as 2,370 µg/l. In the 0-3,000 foot zone, definite effects were noted in the periphyton and macroinvertebrates colonizing artificial substrates. Effects were manifested by shifts in population, number of species, and community structure.

The results of the study indicate no environmental effect on periphyton or macroinvertebrates from a complex TNT manufacturing effluent at a total munitions concentration of less than 25 µg/l. Definite effects were noted during a period when total munitions averaged 500-600 µg/l with a peak of over 2,000 µg/l. Concentrations believed to be in the range of 50-100 µg/l produced minimal effects.

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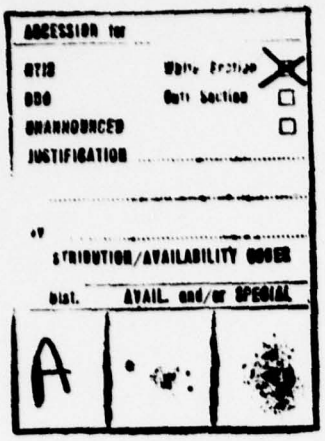

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EXECUTIVE SUMMARY

Field surveys were conducted during December, 1976, and March, 1977, in Waconda Bay to determine the distribution and effect on periphyton and macroinvertebrates of TNT effluent discharged from Volunteer Army Ammunition Plant. The study was an extension of a 1975 summer effort to determine no-effects levels for munitions compounds in fresh water.

The study was divided into two phases. Phase I, completed in December, defined the movement of the effluent in the bay to aid in selection of chemical and biologic sampling sites for the more extensive Phase II work. A dye tracer study utilizing Rhodamine B, in situ, and total munitions measurements were utilized in Phase I. Results showed that dispersion of effluent into the Bay was most influenced by reservoir stage changes. A rising stage had the effect of holding the effluent in the upper part of the Bay while a drawdown period moved effluent rapidly downbay.

During a one-week intensive sampling period, total munitions concentration was highest in the first 3,000 feet from the waste outfall. Mean total levels were as high as 397 µg/l. The principal munitions compounds near the discharge were 2,4-DNT, 2,6-DNT, and 2,4,6-TNT. Analysis of data from this first phase shows that pollutant concentration in the initial 3,000 feet was about 0.5 to 1 times the effluent concentration. Levels diminished rapidly the next 1,600 feet to 0.01 to 0.05 times that of the effluent. Based on results of the December study, stations for Phase II were grouped in high, medium, and low concentration zones. Chemical and biological characterizations were determined for water and sediments. Low stage conditions which continued to prevail during March (Phase II) caused a channel to form, extending about 3,000 feet beyond the outfall.

Daily munitions analysis during the first week in March showed that highest levels occurred consistently in the channel. Average total munitions concentration in this area was 78 µg/l with the principal components being 2,4-DNT, 2,6-DNT, and 2,4,6-TNT. Total munitions concentration in the effluent during this time based on average 24 hour composites, was 89 µg/l. It is apparent that little dilution was occurring in the channel. Concentrations of munitions compounds dropped significantly beyond the channel. Relative to other compounds only three of the individual Bay samples showed 2,4-DNT and five samples contained 1,3-DNB. Only 1,3,5-TNB showed increased levels downbay. It appears that this compound is a breakdown product of 2,4,6-TNT. No 1,3,5-TNB was detected in the plant effluent.

In early March total nitrogen in the channel averaged 14.2 mg/l with approximately 79 percent occurring as NO_3-N. Concentrations downbay showed a gradual reduction. The N levels in the medium zone averaged 5.6 mg/l with nitrate occurring again in the same proportion. Outer bay stations reflected a mean nitrogen content of 4 mg/l with NO_3-N concentration as the most significant form. Other constituents such as chloride, total hardness, and sulfate followed a similar pattern of decreasing concentrations downbay.

For a period of one month while artificial substrates were exposed in Waconda Bay, 24-hour composite samples were collected of VAAP effluent. Results show that average levels during the incubation of biological samplers were higher than during the early March field survey. Total munitions in the channel probably were near 2,000 µg/l at a maximum and averaged 500 µg/l. Organisms in the intermediate zone just beyond the channel were likely exposed to a concentration of 50 - 100 µg/l.

Periphyton and macroinvertebrates were the biological components selected for study. Of these, periphyton analysis provided the most useful information. Community structure was examined following a 30-day colonization of standard glass microscope slides. Suspension units containing up to 8 periphytometers were utilized during incubation. A correlative study examined between slide and within slide variability using population size and species diversity as variables.

The periphyton population density was highly correlated with concentrations of VAAP effluent. Population sizes expressed as no/mm^2 were all less than 1,000 in the narrow channel. As the channel widened where down-bay water mixed and diluted the waste, populations increased two orders of magnitude and remained at this level throughout Waconda Bay. Treatment of the data by the Pinkham-Pearson Similarity Index shows this relationship among stations. Levels of chlorophyll a and organic biomass add support to this latter conclusion. Chlorophyll a on colonized slides at the various stations show a distribution consistent with population counts. Organic biomass follows a similar pattern. However, Shannon-Weaver diversity did not correlate with munitions concentrations. This index appears to be of limited value in situations where the main environmental response is a change in population density.

The statistical analysis indicates that a principal source of variability in estimating either diversity or population size of periphyton colonizing artificial substrates is the difference among direct microscopic field counts within individual slides. Unequal distribution of organisms which occurred during the drying of the aliquot on the glass coverslip produced this variability. Locations near the edge of the coverslip had lower populations than those near the center. The result of an uneven distribution of diatoms is a masking of aliquot and slide to slide variation. The error due to drying on the coverslip must be reduced before a full understanding of periphyton variability can be attained.

Macroinvertebrate distribution in sediments did not reveal clearly defined relationships to TNT waste products. Low populations encountered because of the cold season are considered as the principal reason. Midges which are an abundant form in Waconda Bay colonize mainly during the summer but are sparse during the winter. Species dominating in the March survey were Procladius sp., Coelotanypus spp. and a Chironomus sp. Procladius dominated in the channel nearest to the outfall and may have represented a form tolerant to the waste.

Chironomids also dominated Hester-Dendy artificial substrate samplers. The data show a more defined relationship among stations than is apparent with sediments. Even so the definition is not clear cut. There appears to be maximum impact in the 3,000 foot channel section with a suggestion of transition zones in the outer bay areas. Overall the results suggest that macroinvertebrates are not as useful a parameter to study TNT effects during a winter sampling effort as are periphyton.

It should be noted that the observed correlations between munitions concentrations and biologic responses do not establish precise cause and effect relationships. The complex effluent at VAAP contains many compounds that vary in concentration with regard to the specific munitions-unique compounds measured. These compounds may be factors also in the effects observed.

Nevertheless, based on the winter survey, no biological effects were discerned when total munitions concentration was less than 25 µg/l. Definite effects were noted when munitions levels averaged 500 - 600 µg/l with a peak of around 2,000 µg/l. Minimal effects were observed at munitions levels in the range of 50 - 100 µg/l. These data suggest a no-effects level of 25 µg/l for a complex TNT effluent.

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INTRODUCTION

The Volunteer Army Ammunition Plant (VAAP) is situated on 7,300 acres northwest of Chattanooga, Tennessee and manufactures trinitrotoluene (TNT) on a contractor-operated basis. Wastewater from VAAP drains northward into a series of treatment lagoons and is discharged into the head of Waconda Bay after undergoing pH adjustment with lime. Figure 1 shows Waconda Bay in relationship to Harrison Bay and Lake Chickamauga.

The purpose of this study was to determine the effects of munitions wastes on periphyton and macroinvertebrates during the cold season. The investigation was dual-phased initiated by a dye study coupled with abbreviated environmental sampling. The main objective of the Phase I study was to determine if sufficient concentrations of munitions wastes were present in Waconda Bay to justify more intenstive biologic work and, if so, to select sampling sites so that the full range of munitions concentrations could be evaluated.

The Phase II work included more intensive biologic sampling of periphyton and macroinvertebrate communities utilizing both artificial and natural substrates. This work was originally scheduled for early February but was postponed to early March because Waconda Bay was frozen in January and February. A supplementary study was included in Phase II to determine the statistical variability occurring in population density and community structure of periphyton colonizing glass slides as a function of sampling and processing technique.

Wastewater and sediment characterization was conducted throughout both segments of the field study. Plant effluent samples were also collected and analyzed for TNT and associated transformation products for a one-month period following the second phase field survey. This coincided with the incubation of the artificial substrate samplers.

Differences in reservoir conditions between this work and the prior summer 1975 work included: 1) lower water temperatures, and 2) lower reservoir stage. This latter factor significantly restricted mixing in the upper end of Waconda Bay by confining the flow to a relatively narrow channel for about 3,000 feet.

Midway through this second field survey the Volunteer Plant was closed by the Department of Defense. Although TNT production was stopped, wastes continued to be released into Waconda Bay during the entire period of study.

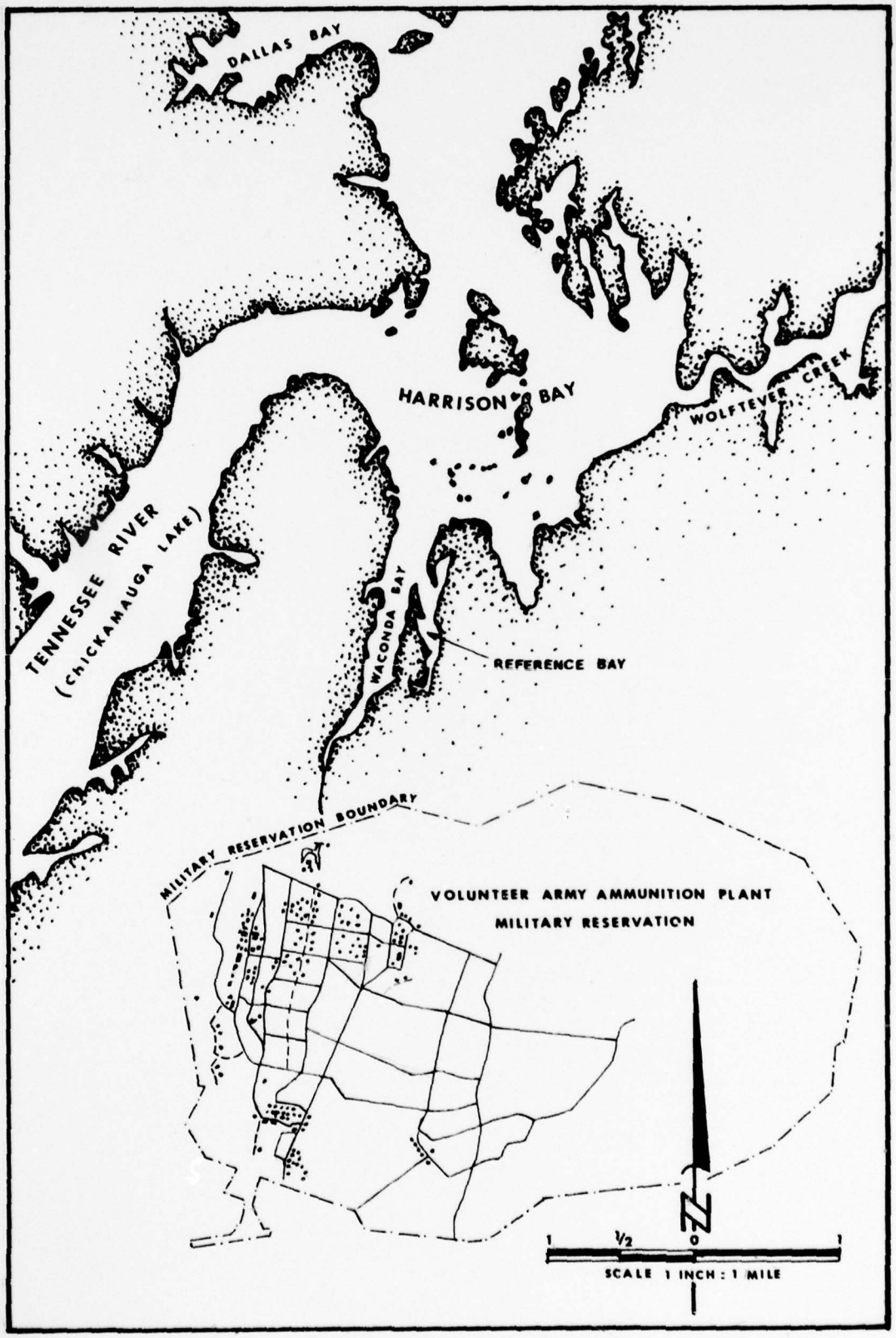

FIGURE 1. VICINITY MAP OF VAAP STUDY AREA.

PHASE I - FIELD SURVEY

The purpose of the December, 1976 field survey was: 1) to conduct a dye tracer study to determine flow and dispersion patterns in Waconda Bay; 2) to make on-site measurements of D.O., temperature, conductivity, and pH; 3) to collect samples of water and sediment for munitions analysis; and 4) to obtain limited samples of benthic and periphyton organisms for familiarization purposes. Based on the information obtained, a decision could be made as to whether or not a sufficient concentration of munitions existed to justify a more intensive biologic survey, and if so, further determine the sampling sites locations in order to encounter the full range of munitions concentrations. The survey was conducted during the period December 6-14, 1976. Daily measurements were made of D.O., temperature, conductivity, and pH for the 5 day period, December 7-11 at nine sites in Waconda Bay. Samples for munitions analysis were taken daily from these nine sites plus the effluent from the last treatment pond. Each munitions sample consisted of a composite of three grabs taken during the day. The sites utilized were the same as used in the summer 1975 work (see Figure 2) with the following exceptions. Site B-1 was not sampled as it was dry land at the low reservoir levels encountered. One sample was taken in the middle of Transect F; Transects S, T, and U were not sampled.

Tabulations of the field-measured D.O., temperature, and conductivity values are shown in Appendix Table A-1. Dissolved oxygen was near saturation values at all stations and temperatures were in the 5-10°C range. High conductivity values were observed at stations near the waste outfall and this parameter appeared to be a good indicator of munitions waste. Equipment failure precluded measurement of pH. Munitions concentrations found in water and sediment samples are tabulated in Tables 1 and 2. The concentrations found exceeded those which in prior field studies have been shown to produce effects in both benthic and periphyton communities (Sullivan, et al., 1977).

The dye tracer study began December 7 when 153 ml of Rhodamine B was placed in the plant effluent from 1030 to 1300 hours. Based on previous experience in other waters, it was felt that a dye concentration of near 50 ppb could be utilized with minimum discoloration of the lake water. Unfortunately, the lake water had a light green tint that contrasted sharply with the red dye making concentrations of 10-20 ppb highly visible. State of Tennessee environmental personnel had been informed and approved of the tracer study. They had requested however, that obvious discoloration be kept to a minimum to limit citizen complaint and/or alarm. Dye feed began at about 32 ppb but was reduced after 20 minutes to 15 ppb because of excess color. Even at the reduced rate, the dye plume in the upper end of Waconda Bay was clearly visible initially. By the following morning, however, maximum dye concentration was down to 2.5 ppb, and was not visually detectable. By mid-afternoon, maximum concentration was 2.0 ppb and most of the dye was within the narrow channel at the upper end of the bay. The rapid decrease in dye concentration observed in one day suggested that the concentration would be below detection limits when it moved further down into a much wider portion of the bay. Since it was desirable to observe the dye further down bay, an additional 116 ml of dye was released into the center of the plume at 1600 hours on December 8. Even with this addition, the maximum concentration found the following morning was 2.0 ppb and the peak concentration was upbay of where it had been the previous afternoon. Transects were made of the dye plume vertically and

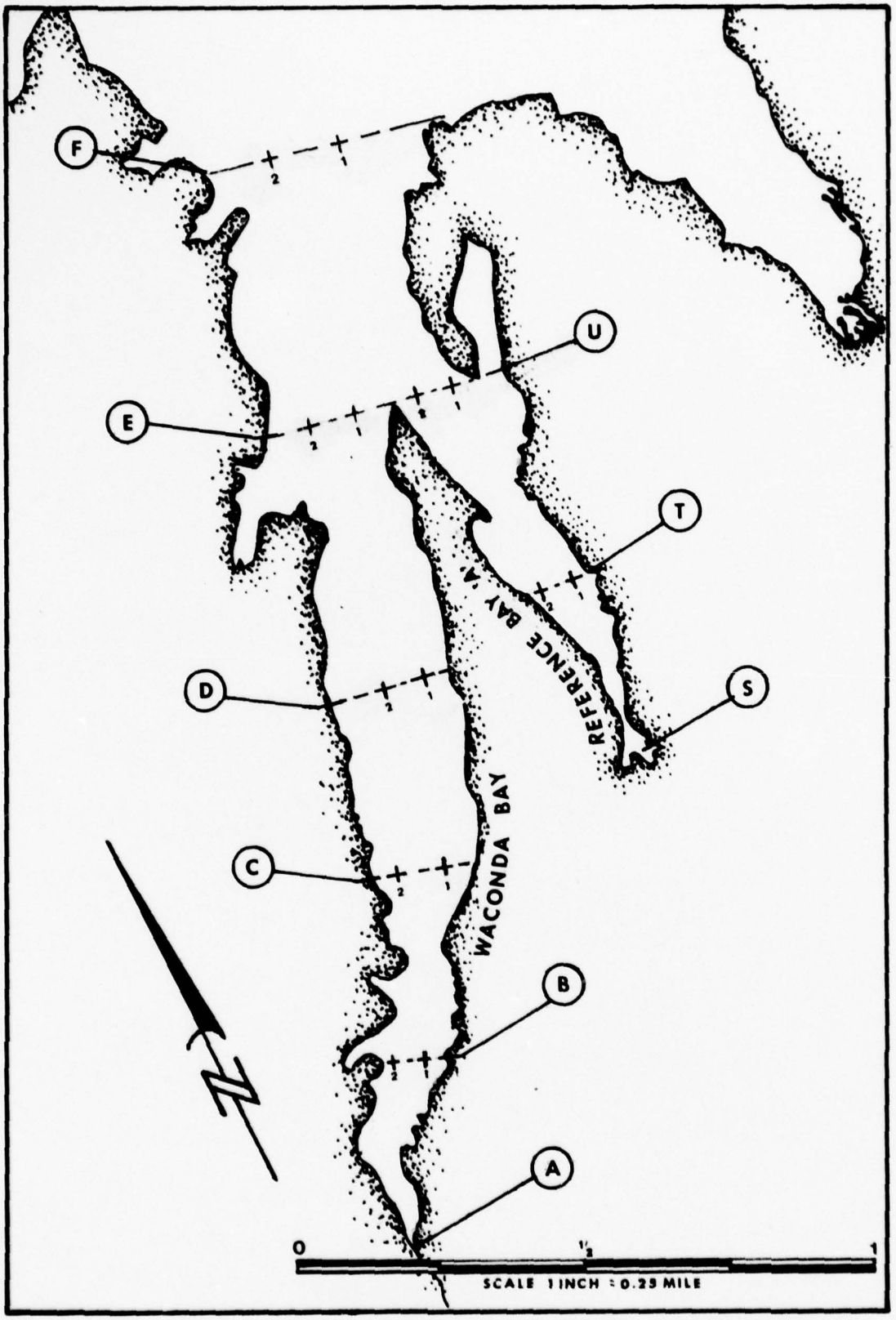

FIGURE 2. SAMPLING STATIONS IN WACONDA BAY AND ADJACENT REFERENCE BAY 'A'. SUMMER, 1975 SURVEY

TABLE 1

WATER MUNITIONS DATA, MEAN VALUES
DECEMBER 1976

STATION	VALUES REPORTED IN µg/l					TOTAL
	2,4-DNT	2,6-DNT	TNT	1,3-DNB	1,3,5-TNB	
EFFLUENT	93	53	47	4	<1	198
A	173	89	128	6	<1	397
B	156	88	110	5	<1	360
C-1	23	18	5	<1	<1	48
C-2	78	40	34	6	<1	159
D-1	9	4	<1	1	2	17
D-2	14	11	<1	<1	2	29
E-1	6	3	<1	<1	<1	≤12
E-2	2	<1	<1	<1	<1	≤ 6
F	2	<1	<1	<1	<1	≤ 6

TABLE 2

SEDIMENT MUNITIONS ANALYSES, DECEMBER 1976

STATION NO.	As Received Basis, ppm				% Moisture	Dry Weight Basis, ppm			
	2,4 DNT	2,6 DNT	2,4,6 TNT	TOTAL		2,4 DNT	2,6 DNT	2,4,6 TNT	TOTAL
A	0.2	0.1	0.4	0.7	77.7	0.9	0.4	1.8	3.1
B	0.15	0.1	0.25	0.5	76.2	0.6	0.4	1.1	2.1
C-1	<0.1	<0.1	<0.1	<0.1	68.5	<0.3	<0.3	<0.3	<0.3
C-2	<0.1	<0.1	0.15	0.15	44.3	<0.2	<0.2	0.3	0.3
D-1	0.1	<0.1	0.15	0.25	27.8	0.1	<0.1	0.2	0.3
D-2	<0.1	<0.1	0.1	0.1	64.6	<0.3	<0.3	0.3	0.3
E-1	<0.1	<0.1	<0.1	<0.1	58.5	<0.2	<0.2	<0.2	<0.2
E-2	<0.1	<0.1	<0.1	<0.1	27.7	<0.1	<0.1	<0.1	<0.1
F	<0.1	<0.1	<0.1	<0.1	66.0	<0.3	<0.3	<0.3	<0.3

Detection limit on As Received Basis - 0.1 ppm for 2,4 DNT, 2,6 DNT, and 2,4,6 TNT

No 1,3 DNB or 1,3,5 TNB detected with a detection limit of about 0.3 ppm

Overall recovery efficiency through extraction and clean-up $\sim$ 80%

Above values are not corrected for recovery efficiency.

horizontally using a continuously measuring and recording fluorometer. The general pattern of dye movement can be seen in Figure A-1.

In an effort to better understand the dye movement, reservoir stage data were obtained from TVA and isoconcentration plots were prepared of the conductivity and total munitions data. These data are shown in Figures A-2 to A-4. Examination of stage data shows that the dye was initially introduced at the end of a rapid drawdown period. From December 7 through December 10, the reservoir stage increased by 1.15 feet. From Transect E upstream this increase represents a volume change of about 43 million gallons. This is more than the total effluent flow from VAAP during this period so net flow was actually upbay at Transect E. This would have the effect of holding effluent in the upper end of the bay. The reservoir level dropped approximately 0.9 feet during the morning of December 11, which had the effect of moving contaminants downbay at an accelerated rate and which the conductivity and total munitions data demonstrate.

Analysis of the combined data shows that concentrations of pollutants in the first 3,000 feet of the bay were roughly 0.5-1.0 times effluent concentrations. This appears to be due to the limited cross-section of the channel at the low reservoir stages seen during the winter months (channel width 100 ft. depth 5-6 ft.) which minimizes mixing with cleaner water in the bay. Concentrations then drop off very rapidly over the next 1600 ft. to levels around 1-5 percent of effluent. Variations in absolute concentrations can be significantly affected by changes in effluent concentrations, flow, and by changes in reservoir stage.

Four sampling sites were selected for "high" concentration ranges in the upper 2500 feet of the bay; three sites were selected in "middle to low" ranges within 1500 feet of the point where the bay significantly widens and; two additional sites were selected further downbay for the extreme low level range. Three sites were chosen as reference points in the adjacent bay. All sites selected for the second phase study are shown in Figure 3.

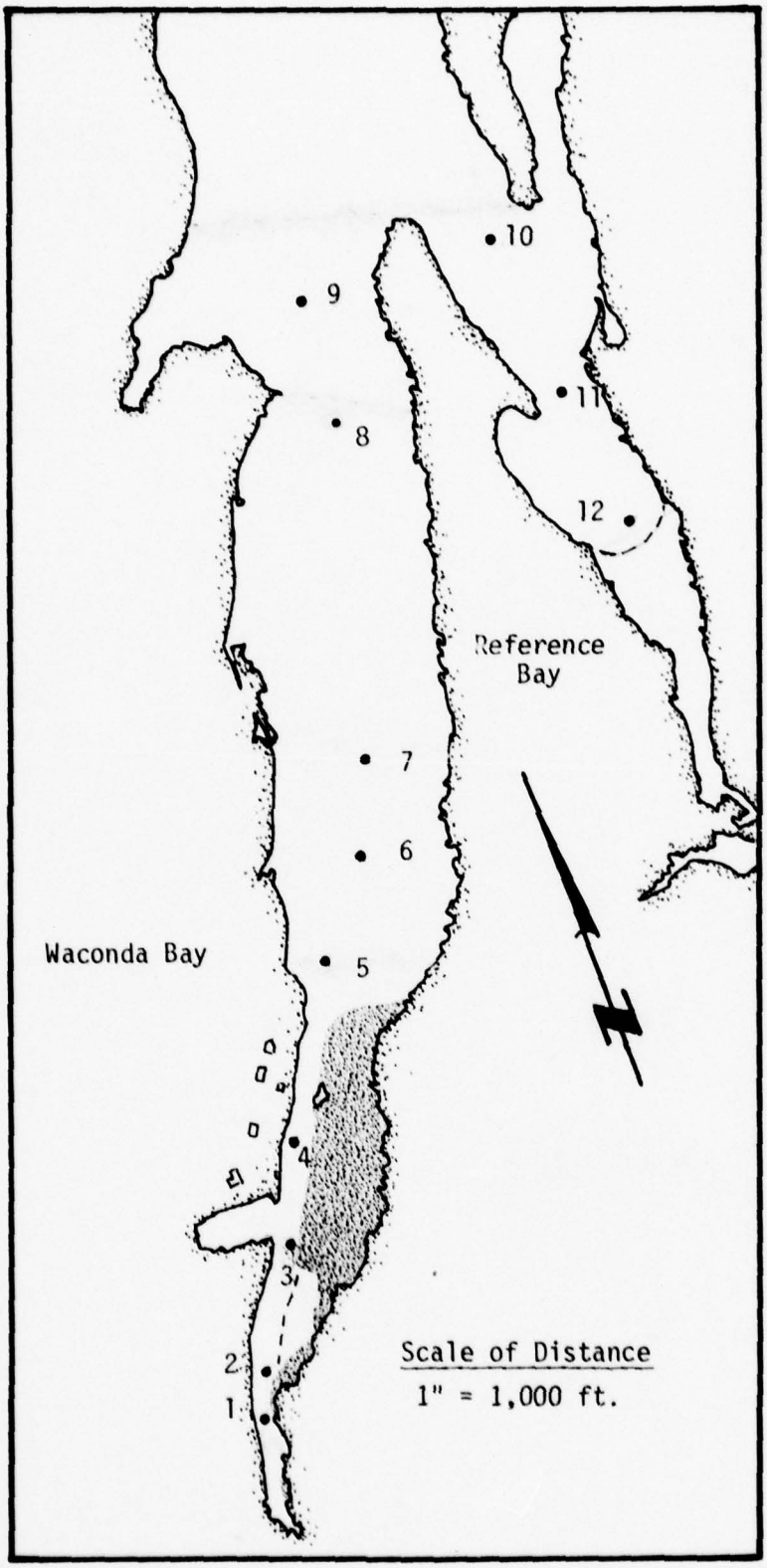

FIGURE 3. SAMPLING SITES FOR PHASE II STUDY

PHASE II - WATER CHEMISTRY

Introduction

During the period March 1-5, 1977, a second field survey was conducted in Waconda Bay. Field data were taken daily for D.O., temperature, and conductivity. Dissolved oxygen was near saturation values at all stations and temperatureswere in the 7-13°C range. High conductivity values were observed at stations near the waste outfall and this parameter appeared to be a good indicator of munitions waste. Equipment failure precluded the measurement of pH. Water samples were taken at each site three times per day and composited. Analyses were made for ammonia-N, total Kjeldahl nitrogen, nitrate-N, chloride, total hardness, sulfate, and munitions. Additionally, effluent samples (24 hour composites) were taken daily during March for munitions analysis.

Sediment samples were taken at each site in Waconda Bay (three samples per site where each sample consisted of three dredge grabs). These samples were analyzed for total and volatile solids, nitrate-N, total Kjeldahl nitrogen, and munitions.

Except for the munitions samples, all analyses were performed in accordance with procedures of Standards Methods (APHA, 1975), (EPA, 1974), or Chemistry Laboratory Manual Bottom Sediments (EPA, 1969). Details of analytical procedures are presented in Appendix B.

Characterization of Water Quality

Mean values for water quality data are shown in Table 3. Typical concentration-distance profiles are shown in Figures 4 through 6 for nitrate-N, ammonia-N, and chloride. For non-munitions parameters, these data show nearly constant average concentrations for stations 1 through 5. Sampling sites 1 through 4 are located in the relatively narrow and shallow channel existing in the upper end of Waconda Bay during low reservoir stages. Station 5 is at the mouth of this channel. The channel geometry in this area limits mixing and dispersion of effluent with other water in the reservoir. Down bay from station 5 the reservoir widens considerably and concentrations decrease rapidly.

Nitrate nitrogen levels were about 11 mg/l at stations 1 through 5 dropping to 1 mg/l at station 9. Ammonia, total Kjeldahl nitrogen, chloride, total hardness, and sulfate also showed elevated levels at stations 1 through 5 with concentrations decreasing from stations 5 to 9.

Five specific munitions related compounds were analyzed: 2,4,6 TNT; 2,4 DNT, 2,6 DNT; 1,3 DNB, and 1,3,5 TNB. The summation of these five compounds versus distance is shown in Figure 7. Mean values for these samples are presented in Table 4. Only three of the individual lake samples showed the presence of 2,4 DNT. Five samples contained 1,3 DNB. This compound was not detected at stations 6 through 9. Most samples contained 2,6 DNT and 2,4,6 TNT and the concentration appeared to decrease from station 5 through 9. By contrast, 1,3,5 TNB was more often found at down bay locations. On only two occasions was this compound detected at stations 1 through 5 compared to thirteen occurrences at stations 6 through 9. It appears that 1,3,5 TNB is a breakdown product, probably of 2,4,6 TNT. Plant effluent samples contained no detectable 1,3,5 TNB or 1,3 DNB.

TABLE 3

MEAN VALUES FOR CHEMICAL ANALYSES OF DAILY SAMPLING DURING THE PERIOD MARCH 1-5, 1977

STATION NUMBER	Mean Values					
	NH_3-N	TKN	NO_3-N	Cl	T. Hard	SO_4
1	1.23	1.91	11.2	17.2	156	155
2	1.21	2.01	11.3	16.9	157	162
3	1.11	1.84	11.5	16.3	161	157
4	1.09	1.87	11.3	15.9	159	163
5	0.95	1.59	11.1	15.2	161	206
6	0.44	0.94	5.59	12.3	125	85.5
7	0.23	0.68	3.24	9.9	107	69.7
8	0.14	0.50	1.89	8.7	92.0	44.2
9	0.07	0.39	1.04	7.8	82.0	21.9
EFF	1.27	1.97	14.1	18.3	162	164
	mgN/l	mgN/l	mgN/l	mgCl/l	mg/l as $CaCO_3$	mgSO_4/l

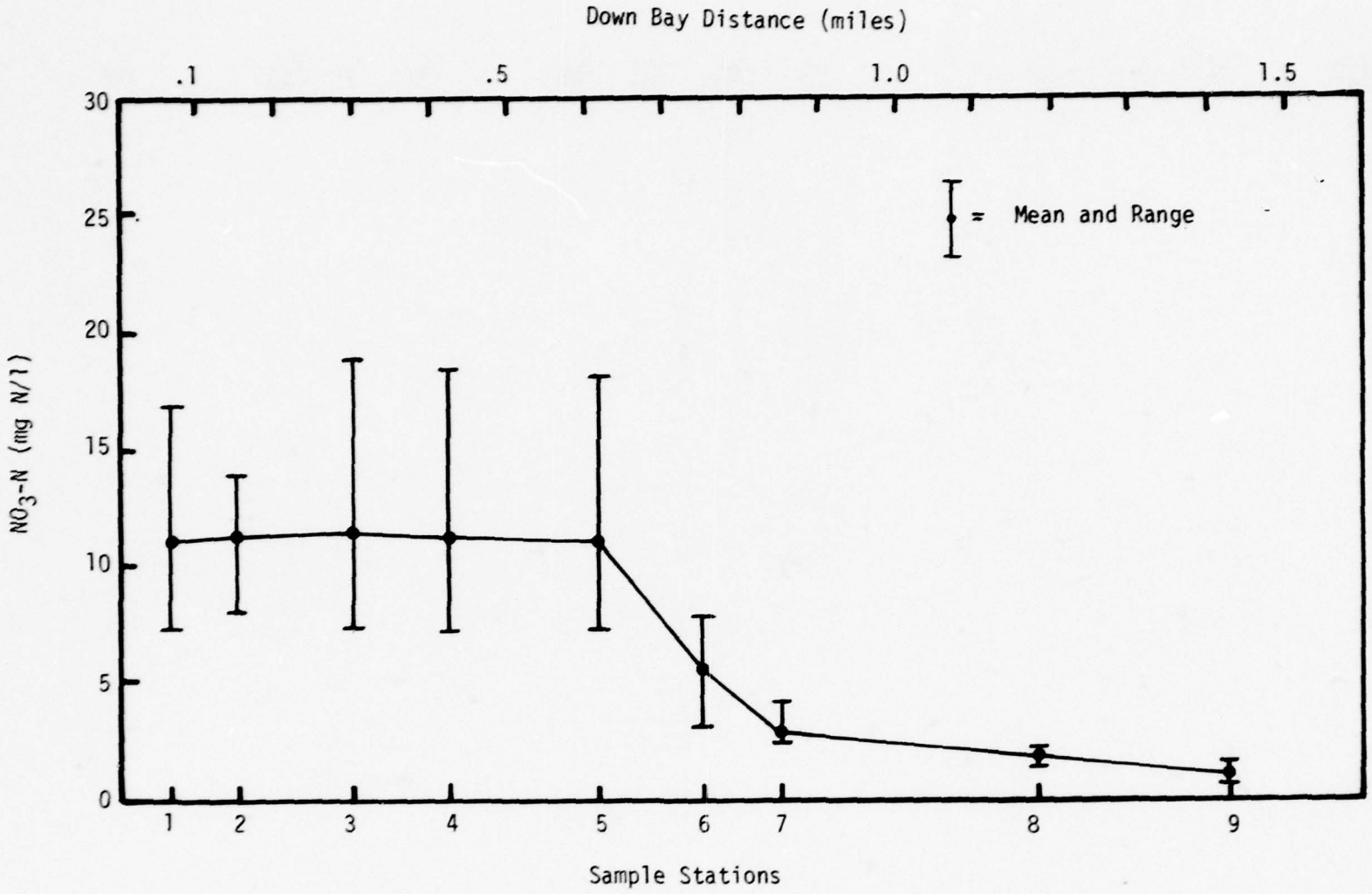

FIGURE 4. NITRATE NITROGEN, MARCH 1-5, 1977

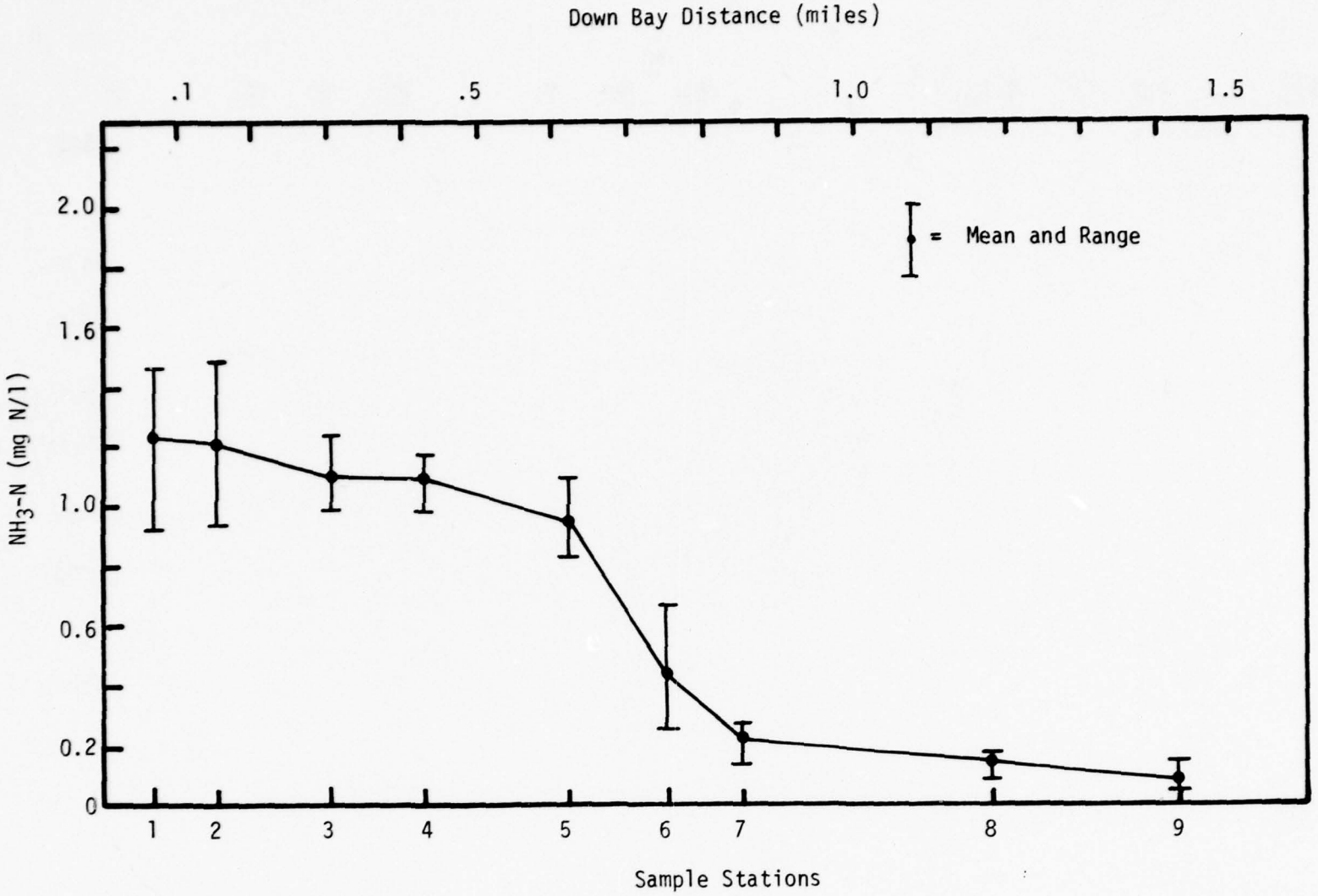

FIGURE 5. AMMONIA NITROGEN, MARCH 1-5, 1977

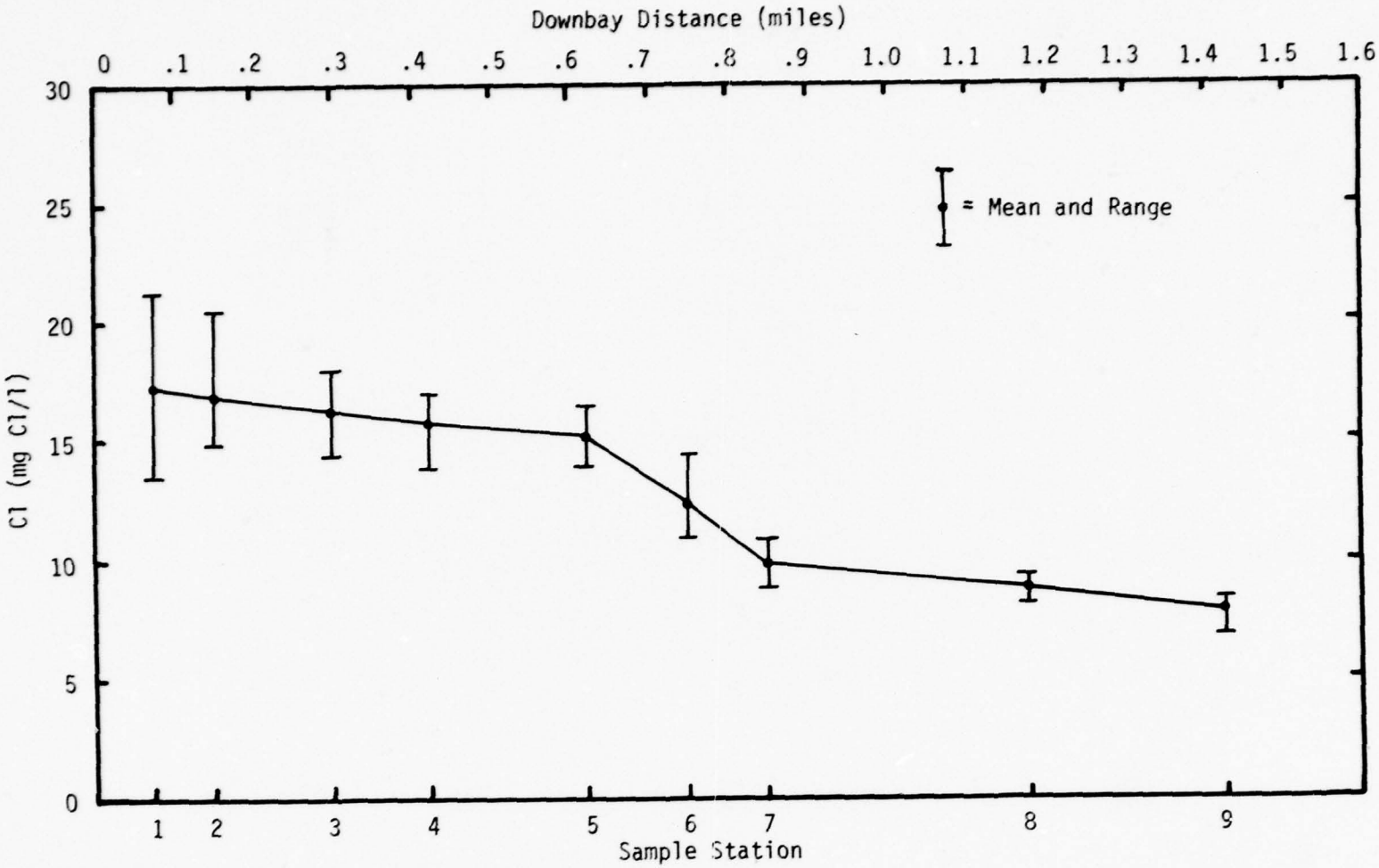

FIGURE 6. CHLORIDE CONCENTRATION, MARCH 1-5, 1977.

TABLE 4

MEAN VALUES FOR MUNITIONS ANALYSES OF DAILY SAMPLING DURING THE PERIOD MARCH 1-5, 1977

STATION NUMBER	MEAN Values in µg/l					
	1,3-DNB	1,3,5-TNB	2,4-DNT	2,6-DNT	2,4,6-TNT	TOTAL
1	4.1	2.9	< 0.10	17.9	48.0	73.0
2	<0.25	<0.75	22.1	38.7	40.3	102
3	<0.25	<0.75	< 0.10	32.0	43.7	76.7
4	0.4	<0.75	17.8	37.2	13.3	69.5
5	0.5	17.1	< 0.10	25.7	26.2	69.7
6	<0.25	1.2	< 0.10	12.1	1.8	≤15.6
7	<0.25	1.6	< 0.10	7.6	0.2	≤ 9.8
8	<0.25	10.6	< 0.10	1.3	0.1	≤12.4
9	<0.25	20.6	1.3	2.3	0.3	24.8
24 hr comp EFF	<0.25	<0.75	28.3	15.5	44.5	89.4

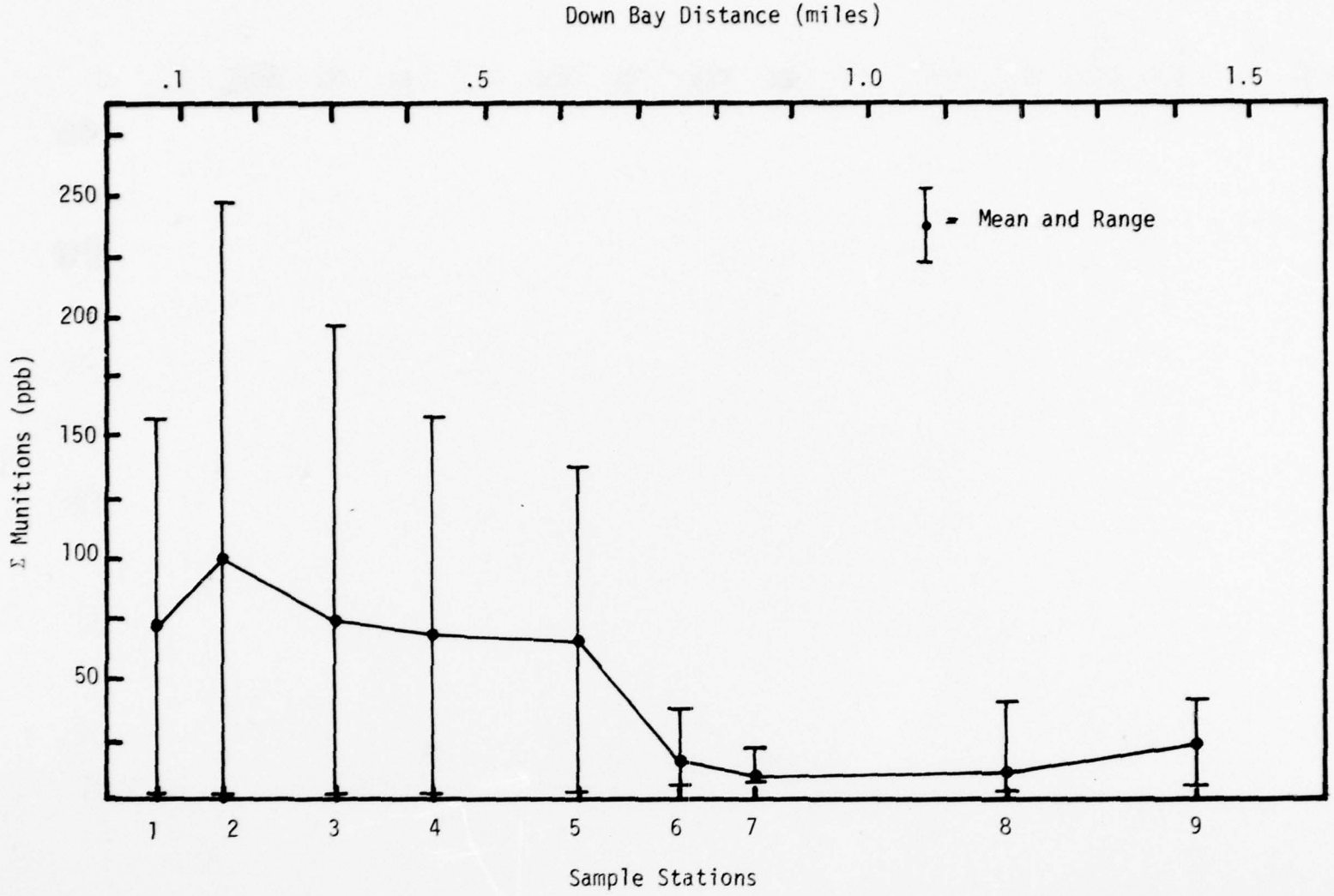

FIGURE 7. TOTAL MUNITIONS, MARCH 1-5, 1977

A station-to-station comparison was made using the following chemical data: NH_3-N, TKN, NO_3-N, Cl, total hardness, SO_4, and total munitions. The data for each parameter was first converted by dividing all values by the maximum value in the set. This puts data between a zero-to-one range. Station-to-station comparisons were made by calculating an average Euclidean distance as follows:

$$\Delta_{AB} \equiv \left[\sum_{i=1}^{n} (X_{iA} - X_{iB})^2 \right]^{\frac{1}{2}}$$

where Δ_{AB} = the Euclidean distance between Stations A and B

n = the number of chemical or chemical/biologic parameters considered

X_{iA} = the magnitude of the i th parameter at Station A

X_{iB} = the magnitude of the i th parameter at Station B

The magnitude of the Euclidean distance, Δ_{AB}, increases for any pair of stations as the number of parameters considered is increased. To eliminate this dependence, an average distance, d_{AB}, may be calculated (Sokal and Sneath, 1963):

$$d_{AB} \equiv \sqrt{(\Delta_{AB})^2/n}$$

where d_{AB} = average distance between Stations A and B

Δ_{AB} = Euclidean distance between Stations A and B

n = number of chemical or chemical/biologic parameters considered

The average Euclidean distance values were used to construct a phenogram showing the relationships between the nine stations (see Appendix for details). This phenogram, Figure 8, shows stations 1 through 5 as decidedly different from stations 6 through 9. The cophenetic correlation coefficient of 0.951 indicates that the phenogram is an accurate representation of the relationship between stations. Generally, coefficients greater than 0.8 are considered to indicate accurate phenograms.

Biologic samplers were placed in Waconda Bay during this survey and remained for a month. During this period samples of plant effluent were taken by VAAP personnel and retained for subsequent munitions analysis. This was done so that the actual munitions concentrations existing during the incubation period could be estimated. The results are shown in Figure 9, and Appendix Table C-1. During the field survey period (March 1-5) the effluent munitions* averaged 89 µg/l. During the following six days effluent munitions concentration generally increased to a maximum of about 2,400 µg/l on March 11. It then decreased to 157 µg/l on March 17. From then until April 1, average effluent concentration was 217 µg/l. With regard to the specific compounds, 1,3 DNB and 1,3,5 TNB were usually below detection limits. For the twenty

* Total of the five specific compounds measured.

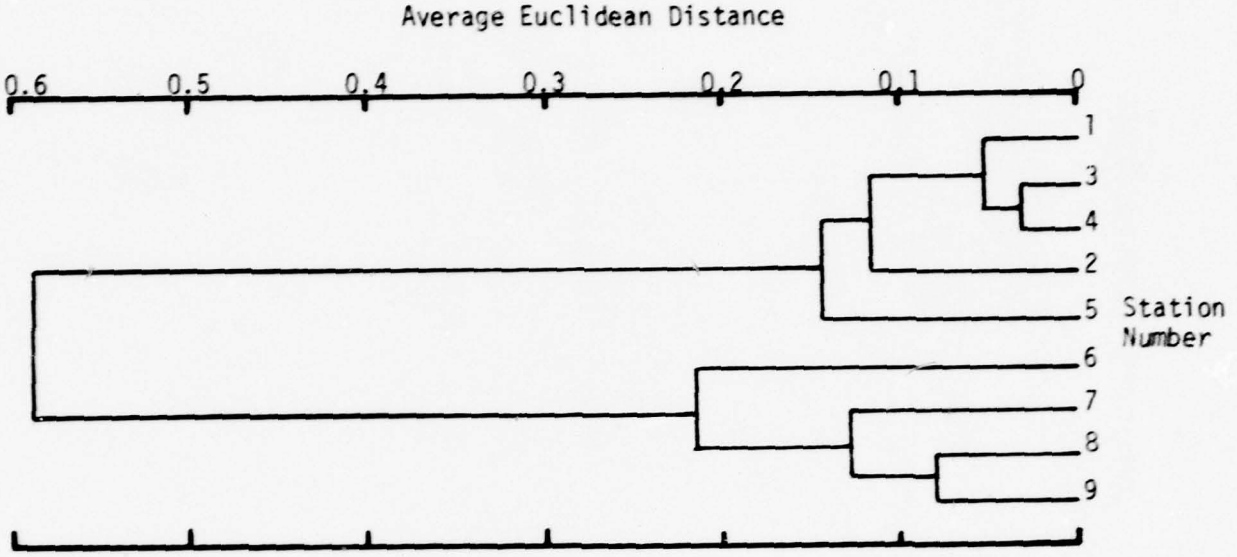

FIGURE 8. PHENOGRAM OF CHEMICAL DATA. COPHENETIC CORRELATION COEFFICIENT 0.951

effluent samples taken from March 5 through April 1, 1977 the concentrations of 2,4 DNT, 2,6 DNT, and 2,4,6 TNT averaged 323, 98, and 151 µg/l respectively. Effluent flow rates remained relatively constant throughout this period averaging 5.9 MGD (see Appendix Table C-2).

Consequently, it appears that average concentrations during the incubation period exceeded those during the field studies. Total munitions in the channel extending down bay to and including station 5 probably approached 2,000 µg/l and averaged near 500 µg/l. Based on the relative munitions concentrations found at station 6 through 9 during the early March survey, total concentrations of munitions may have risen to 50 - 100 µg/l at these sites later in March.

Thus, the munitions concentrations encountered by the organisms colonizing the artificial substrates may have been significantly higher than the concentrations encountered by the natural substrate organisms prior to the early March sampling. No detailed munitions analyses are available for the period preceeding the March sampling.

Characterization of Sediment Quality

Mean values for sediment quality are shown in Table 5. Results for nitrate, total kjeldahl nitrogen, and total solids reveal little except slightly lower TKN values at the first three stations. The munitions results show that 1,3,5 TNB and 2,4,6 TNT clearly predominate 1,3 DNB, 2,4 DNT, and 2,6 DNT. The former compounds were found at all stations and in all except one replicate. For both compounds concentrations at stations 6 through 9 were higher than at stations 1 through 5; 260 and 109 µg/kg for 1,3,5 TNB and 97 and 133 µg/kg for 2,4,6 TNT. At stations 1 and 2 only, 2,4 DNT was found at <10 µg/kg at stations 1 through 5 only, 1,3 DNB and 2,6 DNT were found at <20 µg/kg.

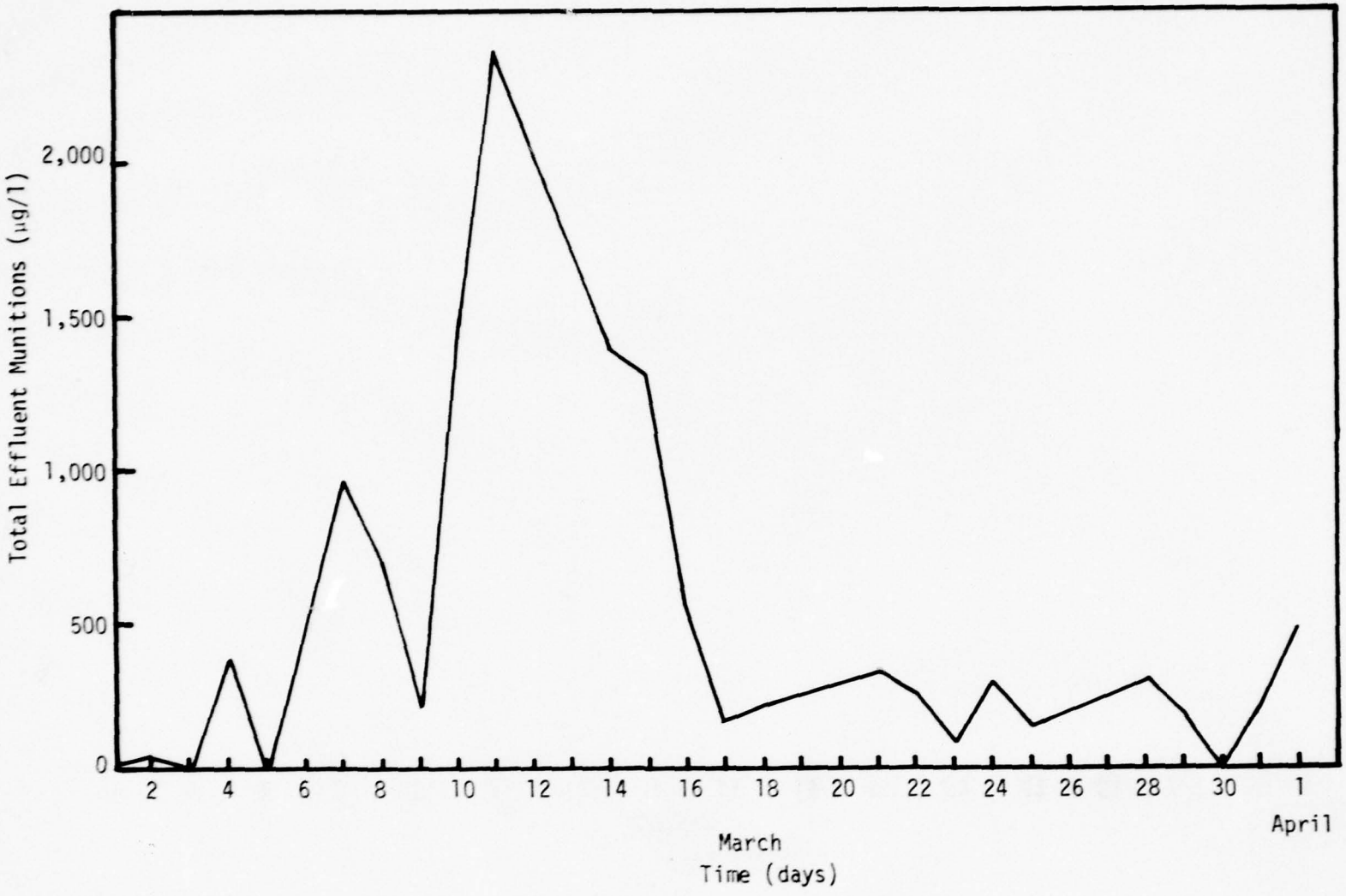

FIGURE 9. TOTAL MUNITIONS CONCENTRATION IN VAAP EFFLUENT DURING MARCH, 1977

TABLE 5

SEDIMENT ANALYSES, MARCH, 1977

STATION NUMBER	NO_2-NO_3	TKN	TS	1,3-DNB	1,3,5-TNB	2,4-DNT	2,6-DNT	2,4,6-TNT
1	≤2	350	14	≤13	73	≤3.4	10	91
2	4	333	12	≤12	≤179	≤7.9	17	119
3	≤2	352	17	≤14	76	<2.5	17	119
4	<2	644	32	11	135	<2.5	≤4.3	86
5	≤5	495	27	14	80	<2.5	11	70
6	<2	628	23	<6.3	221	<2.5	<1.3	138
7	≤11	529	22	<6.3	250	<2.5	<1.3	105
8	<2	466	9	<6.3	304	<2.5	<1.3	142
9	≤2	687	35	<6.3	263	<2.5	<1.3	148
UNITS	mgN/kg	mgN/kg	%	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg

Figures shown are the average of three replicates taken March 3, 1977.

Values reported on a wet weight basis.

PHASE II PERIPHYTON

Introduction

The periphyton or "Aufwuchs" community is an assemblage of attached microorganisms (primarily algae) growing on the surface of submerged substrates and forming a coating which is commonly a green or brown color. This community consists of both autotrophic (i.e. unicellular or filamentous algae) and heterotrophic (bacteria, protozoa, rotifers, ect.) forms.

These organisms are well suited to biological investigations since they remain at fixed locations and are sensitive to environmental alterations. Their populations and biomass are relatively easy to quantify using standard laboratory procedures and are adaptable to a variety of statistical analyses. The results of the survey conducted at VAAP during the winter of 1977 consist of two parts: 1) data pertaining to the field survey and 2) treatment of this information via statistical inference.

The community structure was examined using diatoms and filamentous algae, chlorophyll a, biomass and vital staining. Use of the staining technique showed the live-dead relationship among the periphyton population. The number of diatom frustules that are inactive in a community colonizing a glass slide cannot be estimated using standard techniques. Since the inactive portion may be substantial, an estimate was made on selected stations using tetrazolium violet. The methodology for the various procedures is appended.

Artificial Substrate Colonization Studies

Glass microscope slides with a surface area of 3871 mm^2 were exposed and allowed to colonize for 30 days at the 12 selected stations. Periphytometers containing 8 slide-unit-cartridges were placed together in suspension units as shown in Appendix D. Suspension units containing both 6 and 8 periphytometer units were used in the study. The suspension units with the lower number of periphytometers were used to provide additional slides for the statistical portion of the investigation and were added at those stations predicted to reflect high, medium and low stress from VAAP effluent.

Organism density ranged from 27 per mm^2 at Station 2 to 37,267 at Station 11. Numbers of species per station ranged from 22 to 52 in Waconda Bay and from 76 to 83 in the reference bay. To simplify data presentation and eliminate rare occurring species, a culling routine was completed and all species whose numbers did not constitute at least one percent of the total population at any one station were eliminated. This procedure resulted in a reduction of species from 124 to 30. However, the maximum drop in population density at any one station was 7 percent. Table 6 shows the differences in density and number of species before and after culling. The culled data are shown in Table 7. Organism density is significantly different at stations 1 through 5 as compared to stations 6 through 12 (see statistical report in Appendix).

TABLE 6

A COMPARISON OF POPULATION SIZE AND NUMBERS OF SPECIES
IN CULLED AND UNCULLED DATA AT 1%.
PERIPHYTON - ARTIFICIAL SUBSTRATE

	STATION 1	2	3	4	5	6
CULLED						
no/mm^2	135	27	76	165	727	14721
species	24	22	25	25	25	18
UNCULLED						
no/mm^2	141	27	78	169	745	14916
species	39	22	46	46	52	37
% DIFFERENCE						
no/mm^2	4	0	3	2	2	1
species	38	0	46	46	52	51

	STATION 7	8	9	10	11	12
CULLED						
no/mm^2	18714	27021	21970	-	35158	18354
species	21	25	22	-	22	24
UNCULLED						
no/mm^2	18941	27785	22458	-	37267	19696
species	40	52	39	-	76	83
% DIFFERENCE						
no/mm^2	1	3	2	-	6	7
species	48	52	44	-	71	71

TABLE 7

VAAP PERIPHYTON, ARTIFICIAL SUBSTRATE, MARCH 1977

Taxonomic Classification	Number of Organisms per mm^2 at Station: 1	2	3	4	5	6	7	8	9	11	12
BACILLARIOPHYTA (DIATOMS)											
ACHNANTHES LANCEOLATA V DUBIA	P	2	P	-	P	-	-	8	-	42	123
ACHNANTHES MINUTISSIMA	15	2	9	5	6	291	621	2198	3664	7172	3812
AMPHORA VENETA	-	P	-	P	P	-	-	-	-	-	4
COCCONEIS PLACENTULA V EUGLYPTA	-	-	-	3	-	-	-	-	-	-	-
CYMBELLA PROSTRATA V AUERSWALDII	4	-	2	2	8	69	222	911	1104	2538	1026
DIATOMA TENUE V ELONGATUM	53	2	18	72	511	12987	14137	11218	6194	2828	562
EUNOTIA CURVATA	-	P	-	P	-	-	-	-	-	-	-
FRAGILARIA CAPUCINA	3	-	1	P	7	77	161	502	414	4335	1870
FRAGILARIA VAUCHERIAE	14	1	4	4	24	330	1311	5170	4952	9327	2909
GOMPHONEMA ANGUSTATUM	P	-	2	2	P	-	-	17	-	511	341
GOMPHONEMA PARVULUM	9	5	6	16	19	23	15	59	61	1397	2572
MELOSIRA GRANULATA	7	2	2	7	18	107	130	187	92	400	209
MELOSIRA ITALICA	3	3	3	4	11	8	31	68	15	187	98
MELOSIRA VARIANS	3	-	-	P	6	69	276	1065	1441	579	153
NAVICULA CRYPTOCEPHALA	P	2	2	P	6	-	31	102	77	417	456
NITZSCHIA ACCOMODATA	P	P	P	-	-	-	-	8	-	-	-
NITZSCHIA DISSIPATA	1	P	P	P	1	8	38	366	490	511	464
NITZSCHIA FRUSTULUM V SUBSALINA	1	P	2	3	5	-	31	34	77	93	179
NITZSCHIA KUETZINGIANA	2	P	1	3	14	38	46	85	31	400	370
NITZSCHIA PALEA	P	P	P	2	P	8	46	25	61	-	-
PINNULARIA OBSCURA	-	P	P	-	-	-	-	-	-	-	-
PINNULARIA RUPESTRIS	-	P	-	-	-	-	-	-	-	-	4
STEPHANODISCUS ASTRAEA V. MINUTULA	1	-	P	-	P	23	23	51	15	85	17
STEPHANODISCUS INVISITATUS	1	P	2	2	11	46	123	255	261	443	115
SURIRELLA OVATA	4	5	11	13	10	8	8	25	15	68	266
SYNEDRA PULCHELLA	-	P	P	2	3	-	-	8	15	-	-
SYNEDRA DELICATISSIMA	1	-	P	1	4	46	253	153	169	17	21
SYNEDRA RUMPENS	6	P	4	P	11	560	989	3595	2223	2087	975
SYNEDRA RUMPENS V FAMILIARIS	5	1	5	19	45	-	92	690	322	1414	1491
SYNEDRA ULNA	P	-	P	2	3	23	130	221	307	307	115
TOTAL NUMBER OF ORGANISMS	135	27	76	165	727	14721	18714	27021	21970	35158	16354
NUMBER OF TAXA	24	22	25	25	25	18	21	25	22	22	24

Culled at 1% Level

The dominant species in Waconda Bay are:

Achnanthes minutissima
Cymbella prostrata
Diatoma tenue var. elongatum
Fragilaria vaucheriae
Melosira varians
Synedra rumpens

These species reached population levels of at least 1,000 individuals/ mm^2 at one or more stations in Waconda Bay. Diatom populations were reduced in the impact area from stations 1 to 5 although the actual number of species was approximately the same as down bay.

The marked population increase at station 6 can be correlated with the water chemistry, which, during the March 1-5 survey, showed a significant drop in concentration of several components between stations 5 and 6. (See chemistry section.) Considering the dominants, the ratio of density for dominant organisms between stations 6 and 5 where the Bay widens is as follows:

Achnanthes minutissima	- 48
Diatoma tenue	- 25
Fragilaria vaucheriae	- 14
Melosira varians	- 9
Synedra rumpens	- 51

Figure 10 shows the clustering of stations by the Pinkham-Pearson Index using culled data with mutual absence important. The phenogram shows that stations 1 through 5 are decidedly dissimilar from stations 6 through 12. Additionally, the reference bay stations, 11 and 12, form a sub-group somewhat different from Waconda Bay stations 6 through 9.

Shannon-Weaver diversity values are shown in Table 8. Theoretically, the higher the diversity the "healthier" the community. However, examination of the results shows that diversity in the area of maximum stress, stations 1 to 5, is higher than at the downbay stations 6 to 9. This anomolous condition is caused by the severe drop in population density of all dominant species at stations 1 to 5 which results in a condition of better balance between the surviving species. Since diversity considers only relative proportions of species and ignores absolute population density, it seems to be of limited value in a situation where the main input is seen as reduction in population density.

Natural Substrate Colonization Studies

Natural substrate collections were made at 9 stations in Waconda Bay and 3 stations in the reference bay. A description of these samples and the

PAGE 33 - FIGURE 10 is also PAGE 167 - FIGURE G-1

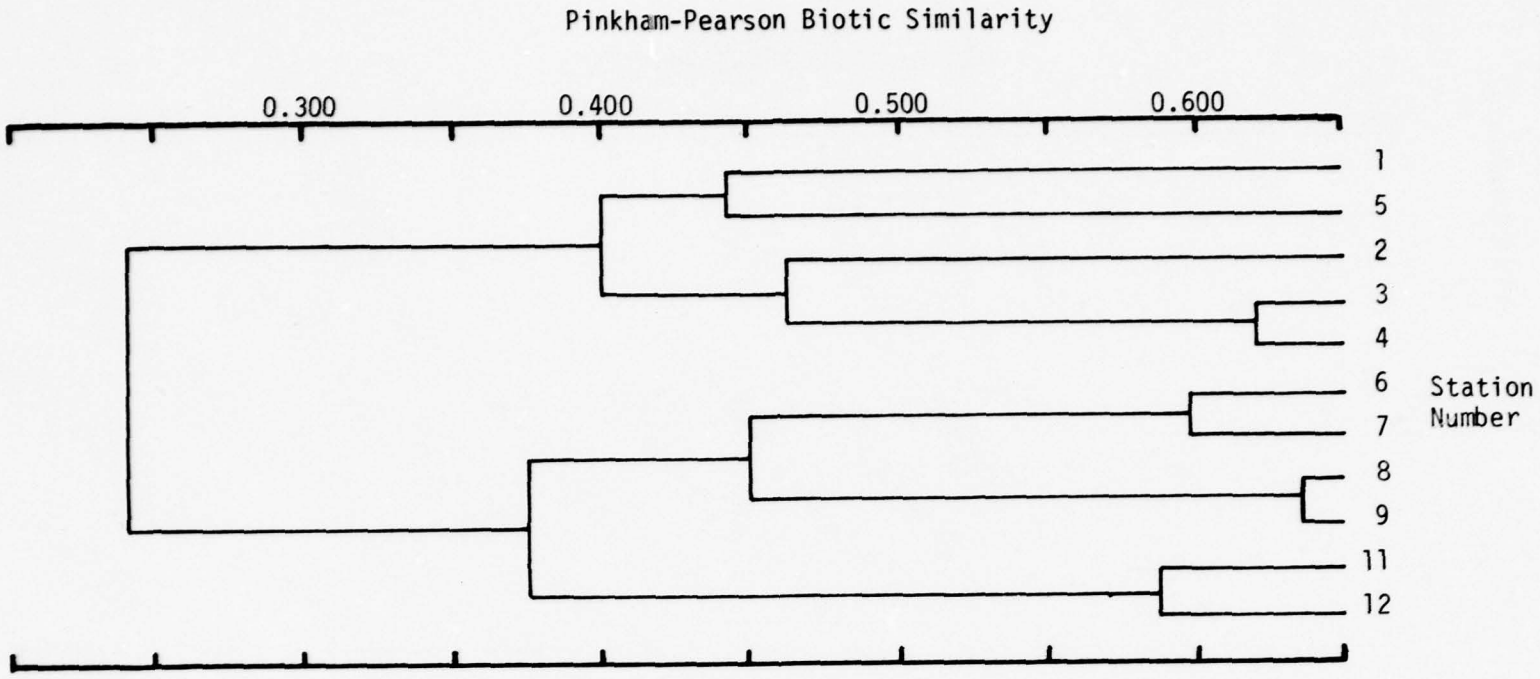

FIGURE 10. PHENOGRAM OF PERIPHYTON, ARTIFICIAL SUBSTRATE, CULLED 1%, MUTUAL ABSENCE IMPORTANT, COPHENETIC CORRELATION COEFFICIENT .871.

TABLE 8

SHANNON-WEAVER DIVERSITY INDICES ($\bar{H}$) FOR VAAP ARTIFICIAL SUBSTRATE DIATOMS, MARCH, 1977

STATIONS	MAGNITUDE culled	MAGNITUDE unculled
1	2.22	2.37
2	2.49	2.49
3	2.49	2.56
4	2.12	2.21
5	1.40	1.54
6	0.60	0.70
7	1.10	1.18
8	1.88	2.03
9	2.04	2.16
10	-	-
11	2.27	2.54
12	2.46	2.78

Base e

resulting data are shown in Table 9 and 10. Generally scrapings were made from submerged branches, rocks, and styrofoam floats. Three substrates were sampled at each location except stations 4 and 8. Counts were made to approximate 500 diatom valves. Comparisons among stations can only be made relative to numbers of individual species colonizing these substrates. The overall total number of species ranged from 11 to 77 with no marked differences related to station locations. In fact, the two extreme values both occurred at station 3. The data are culled to eliminate species that don't make up at least one percent of the total at at least one station.
The five most commonly occurring species were:

1) Achnanthes minutissima
2) Fragilaria capucina
3) Fragilaria vaucheriae
4) Stephanodiscus invisitatus
5) Synedra rumpens

In considering the distribution of individual species it is interesting to note that Achnanthes minutissima was as common an occurence in the impact zone as in other areas of Waconda Bay and the reference bay. This suggests that Achnanthes is not as susceptible to munitions waste as might be expected. In an earlier study conducted during the summer of 1975, Achnanthes minutissima showed a similar response and accounted for 80 percent of the diatom population colonizing artificial substrates in areas of severe stress from VAAP waste. The two Fragilaria sp. reached a maximum of approximately one fifth the population. These levels were attained only at downbay stations. With the exception of Synedra rumpens at station 4, the minor dominants were severely growth restricted in the high stress zone between stations 1 through 5.

Pinkham-Pearson comparisons among the various samples are shown in Figure 11. Clustering of stations using natural substrates results in a grouping based primarily on substrate type rather than location relative to the waste discharge. For example, the first station cluster, with one exception, has a common denominator of wood scrapings as a substrate type. The second, beginning with 4AR includes predominately rock scrapings. Styrofoam substrates principally compose the next group and include samples from stations 6 through 8, 11 and 12. Treatment of the data by the Pinkham-Pearson analysis is especially useful in this case to point out the substrate dependence of periphyton diatoms. The phenographic display in this instance further demonstrates the utility of uniform substrates for periphyton investigations. The cophenetic correlation coefficient of 0.734, however, indicates that caution should be exercised in interpreting this phenogram.

Filamentous Algae

Filamentous forms colonizing glass slides were restricted to green and blue-green species. The data are shown in Table 11. Six genera predominated with the blue-green, Schizothrix calcicola, the most abundant organism at all stations. The second dominant, Oscillatoria sp., was observed at all sampling locations but occurred only sporadically between stations 1 through 5. An Anabaena species was recorded only once from station 12 in the reference area.

TABLE 9

INVENTORY OF NATURAL PERIPHYTON SUBSTRATE
SAMPLES COLLECTED FEBRUARY 28, 1977

Station	Substrate Description
Waste Pond Spillway	Rock Scraping
Waste Outfall Stream	Rock Scraping
"Old Station A"*	Branch Scraping
"Old Station A"	Branch Scraping
"Old Station A"	Branch Scraping
S-1	Branch Scraping^{W}
S-1	Branch Scraping^{W}
S-1	Log Scraping^{W}
S-2	Branch Scraping^{W}
S-2	Branch Scraping^{W}
S-2	Branch Scraping^{W}
S-3	Rock Scraping^{R}
S-3	Branch Scraping^{W}
S-3	Rock Scraping^{R}
S-4**	Dock Scraping^{W}
S-5	Rock Scraping^{R}
S-5	Rock Scraping^{R}
S-5	Rock Scraping^{R}
S-6	Stump Scraping^{W}
S-6	Rock Scraping^{R}
S-6	Dock Styrofoam^{S}
S-7	Dock Styrofoam^{S}
S-7	Dock Styrofoam^{S}
S-7	Branch Scraping^{W}
S-7	Stump Scraping^{W}
S-8	Styrofoam from "No Wake" Marker^{S}
S-8	Scraping from Metal Buoy^{M}
S-9	Scraping of Metal Buoy^{M}
S-9	Rock Scraping^{R}
S-9	Rock Scraping^{R}
S-10	Dock Styrofoam^{S}
S-10	Rock + Old Bottle Scraping^{R}
S-10	Rock Scraping^{R}
S-10	Surface Sediment^{B}
S-11	Wood Dock^{W}
S-11	Dock Styrofoam^{S}
S-11	Rock Scraping^{R}
S-12	Rock Scraping^{R}
S-12	Dock Styrofoam^{S}
S-12	Branch Scraping^{W}

*20 yards from VAAP outfall

**Only 1 substrate available at Station S-4

W = Wood; R = Rock; S = Styrofoam; M = Metal; B = Surface Sediment

TABLE 10

VAAP PERIPHYTON, NATURAL SUBSTRATE, CULLED AT 1%, MARCH 1977

Taxonomic Classification	Number of Organisms at station: 1AN	1BN	1CN	4AN	4BN	4CN	3AN
BACILLARIOPHYTA (DIATOMS)							
ACHNANTHES EXIGUA V HETEROVALVE	-	-	-	-	-	-	-
ACHNANTHES LANCEOLATA V DUBIA	-	-	-	-	-	-	-
ACHNANTHES MINUTISSIMA	193	214	402	361	370	401	69
ACHNANTHES NULLII	-	-	-	2	-	-	-
ANOMOEONEIS VITREA	-	-	1	-	-	1	-
ASTERIONELLA FORMOSA	1	-	-	-	-	-	-
CALONEIS HYALINA	1	-	1	-	-	-	-
COCCONEIS PLACENTULA V EUGLYPTA	1	3	-	-	1	2	7
CYCLOTELLA ATOMUS	-	-	-	-	-	-	-
CYCLOTELLA MENEGHINIANA	1	-	-	1	-	-	10
CYCLOTELLA STELLIGERA	6	-	1	2	-	-	10
CYMBELLA AFFINIS	-	-	-	-	-	-	-
CYMBELLA DELICATULA	-	-	-	-	-	-	-
CYMBELLA LAEVIS	-	-	-	1	-	-	-
CYMBELLA MICROCEPHALA	-	-	-	-	-	-	-
CYMBELLA PROSTRATA V AUERSWALDII	5	1	1	2	12	7	16
CYMBELLA TUMIDA	-	-	1	-	-	-	-
DIATOMA TENUE V ELONGATUM	-	-	-	-	-	-	-
EUNOTIA PECTINALIS V MINOR	-	1	-	-	7	1	1
FRAGILARIA CAPUCINA V MESOLEPTA	-	-	-	-	1	2	-
FRAGILARIA CAPUCINA	-	-	1	-	-	-	-
FRAGILARIA CONSTRUENS N	-	-	-	-	-	-	-
FRAGILARIA CONSTRUENS V VENTER	-	-	-	-	-	-	-
FRAGILARIA PINNATA	-	1	-	-	1	-	3
FRAGILARIA VAUCHERIAE	1	-	-	-	-	-	1
GOMPHONEMA ANGUSTATUM	-	-	-	-	-	-	-
GOMPHONEMA PARVULUM	9	6	6	7	12	6	-
MELOSIRA DISTANS	18	11	1	1	2	2	12
MELOSIRA GRANULATA	10	8	2	1	6	2	12
MELOSIRA ITALICA	48	44	2	1	6	2	12
MELOSIRA VARIANS	2	-	1	-	-	-	1
NAVICULA CAPITATA V HUNGARICA	-	-	-	-	-	-	-
NAVICULA CINCTA	-	-	-	-	1	-	5
NAVICULA CONFERACEA V PEREGRINA	1	1	-	-	-	-	7
NAVICULA CONTENTA V BICEPS	-	-	-	-	-	-	-
NAVICULA CRYPTOCEPHALA	12	4	3	4	3	4	9
NAVICULA MENISCULUS V UPSALIENSIS	2	-	-	-	-	-	-
NAVICULA MINIMA	14	3	3	1	-	-	14
NAVICULA MUTICA	-	-	-	-	-	-	1
NAVICULA PELLICULOSA	2	-	-	-	-	-	-
NAVICULA PUPULA V MUTATA	1	-	-	-	1	1	1
NAVICULA RADIOSA N	-	-	-	-	-	-	-
NAVICULA RADIOSA V TENELLA	4	1	2	1	-	1	15
NAVICULA RHYNCOCEPHALA V GENNANI	-	-	-	-	-	-	-
NAVICULA SALINARUM V INTERMEDIA	-	-	-	-	-	-	-
NAVICULA SECRETA V APICULATA	20	16	-	8	3	4	38
NAVICULA SEMINULUM	-	-	-	-	-	-	-
NAVICULA CF SIMULA	-	-	-	-	-	-	-
NAVICULA SUBHAMULATA	-	-	-	-	-	-	-
NAVICULA SUBTILISSIMA	1	-	-	-	1	1	2
NAVICULA THIENEMANNII	3	2	-	-	1	1	2
NAVICULA SP 6	-	-	-	-	-	-	-
NITZSCHIA ACCOMODATA	-	-	-	-	-	-	-
NITZSCHIA ACCOMODATA	23	40	1	2	2	-	2
NITZSCHIA AMPHIBIA	-	-	-	-	-	-	-
NITZSCHIA CITA	5	-	-	1	-	1	2
NITZSCHIA DISSIPATA	3	2	5	1	1	-	2
NITZSCHIA DENTICULA	7	-	-	-	-	-	-
NITZSCHIA FILIFORMIS	4	10	2	2	2	1	7
NITZSCHIA FRUSTULUM V SUBSALINA	-	11	8	2	2	4	15
NITZSCHIA KUETZINGIANA	24	37	13	15	7	1	12
NITZSCHIA PALEA	24	12	6	12	2	1	14
NITZSCHIA RECTA	-	-	-	-	-	-	-
NITZSCHIA SINUATA V TABELLARIA	2	1	-	-	1	1	2
NITZSCHIA SP 9	-	-	-	-	-	-	-
STEPHANODISCUS ASTRAEA	-	-	-	-	-	-	-
STEPHANODISCUS ASTRAEA V. MINUTULA	13	7	2	2	1	3	10
STEPHANODISCUS INVISITATUS	14	23	5	20	1	3	10
SURIRELLA OVATA	7	2	5	1	-	-	6
SURIRELLA SUECICA	-	-	-	-	-	-	-
SYNEDRA DELICATISSIMA	3	1	1	-	-	-	1
SYNEDRA RUMPENS	23	12	1	2	20	15	10
SYNEDRA RUMPENS V FAMILIARIS	3	2	-	-	1	1	1
SYNEDRA ULNA	-	-	-	-	-	-	-
TABELLARIA FLOCCULOSA	-	-	-	-	-	-	-
TOTAL NUMBER OF ORGANISMS	481	483	476	492	491	490	480
NUMBER OF TAXA	40	30	26	34	29	29	44

Taxonomic Classification	Number of Organisms at station:						
	3BR	3CR	4AR	5AR	5BR	5CR	6AR
BACILLARIOPHYTA (DIATOMS)							
ACHNANTHES EXIGUA V HETEROVALVE	-	-	-	2	4	12	1
ACHNANTHES LANCEOLATA V DUBIA	-	1	-	-	14	7	-
ACHNANTHES MINUTISSIMA	425	477	203	32	105	57	169
ACHNANTHES NULLII	-	-	-	2	-	-	-
ANOMOEONEIS VITREA	-	-	1	-	-	-	2
ASTERIONELLA FORMOSA	-	-	1	1	1	1	-
CALONEIS HYALINA	1	-	1	-	20	11	-
COCCONEIS PLACENTULA V EUGLYPTA	-	-	-	-	12	-	-
CYCLOTELLA ATOMUS	-	-	-	2	-	-	3
CYCLOTELLA MENEGHINIANA	-	-	1	2	-	3	1
CYCLOTELLA STELLIGERA	-	-	5	2	-	3	7
CYMBELLA AFFINIS	-	-	-	-	-	-	-
CYMBELLA DELICATULA	-	-	-	-	1	-	-
CYMBELLA LAEVIS	-	-	-	1	-	-	-
CYMBELLA MICROCEPHALA	-	-	-	-	1	-	2
CYMBELLA PROSTRATA V AUERSWALDII	-	1	3	3	1	1	13
CYMBELLA TUMIDA	-	-	-	-	-	-	-
DIATOMA TENUE V ELONGATUM	-	-	25	35	2	27	16
EUNOTIA PECTINALIS V MINOR	6	15	1	1	27	17	-
FRAGILARIA CAPUCINA V MESOLEPTA	-	-	-	-	-	-	1
FRAGILARIA CAPUCINA	-	-	1	49	10	8	14
FRAGILARIA CONSTRUENS N	-	-	-	-	-	-	-
FRAGILARIA CONSTRUENS V VENTER	-	-	-	1	-	1	-
FRAGILARIA PINNATA	-	-	-	-	-	-	-
FRAGILARIA VAUCHERIAE	-	-	14	12	1	14	14
GOMPHONEMA ANGUSTATUM	-	-	-	-	1	-	-
GOMPHONEMA PARVULUM	-	7	5	3	14	17	1
MELOSIRA DISTANS	-	-	9	10	2	1	5
MELOSIRA GRANULATA	-	1	3	40	12	5	14
MELOSIRA ITALICA	3	2	11	40	4	9	14
MELOSIRA VARIANS	-	-	-	-	-	-	-
NAVICULA CAPITATA V HUNGARICA	1	-	-	3	1	1	2
NAVICULA CINCTA	-	-	-	-	2	2	5
NAVICULA CONFERACEA V PEREGRINA	-	-	2	2	-	-	-
NAVICULA CONTENTA V BICEPS	-	-	-	-	-	-	-
NAVICULA CRYPTOCEPHALA	3	-	7	14	17	1	16
NAVICULA MENISCULUS V UPSALIENSIS	-	-	-	5	3	1	-
NAVICULA MINIMA	4	-	-	1	134	199	2
NAVICULA MUTICA	6	-	-	1	-	-	-
NAVICULA PELLICULOSA	-	-	-	-	-	-	-
NAVICULA PUPULA V MUTATA	-	-	-	1	1	1	-
NAVICULA RADIOSA N	-	-	-	-	-	-	-
NAVICULA RADIOSA V TENELLA	-	-	-	-	-	-	-
NAVICULA RHYNCOCEPHALA V GERMANI	-	-	2	24	2	-	2
NAVICULA SALINARIUM V INTERMEDIA	-	-	-	-	-	-	-
NAVICULA SECRETA V APICULATA	12	-	7	31	18	14	13
NAVICULA SEMINULUM	1	-	-	1	2	10	-
NAVICULA CF SIMULA	-	-	-	-	-	-	-
NAVICULA SUBHAMULATA	-	-	-	-	-	-	-
NAVICULA SUBTILISSIMA	2	2	1	86	-	2	52
NAVICULA THIENEMANNII	-	-	-	-	-	-	-
NAVICULA SP 6	-	-	-	-	-	-	-
NITZSCHIA ACCUMODATA	-	-	1	-	-	-	1
NITZSCHIA ACCUMODATA	-	-	-	-	-	-	-
NITZSCHIA AMPHIBIA	-	-	-	-	-	-	-
NITZSCHIA HITA	-	-	1	20	1	20	2
NITZSCHIA DISSIPATA	-	-	1	-	-	-	-
NITZSCHIA DENTICULA	1	-	1	3	36	-	-
NITZSCHIA FILIFORMIS	-	-	-	-	-	-	-
NITZSCHIA FRUSTULUM V SUBSALINA	5	-	1	10	2	-	-
NITZSCHIA KUETZINGIANA	1	-	23	13	8	17	65
NITZSCHIA PALEA	-	-	-	13	-	5	-
NITZSCHIA RECTA	-	-	-	-	-	-	-
NITZSCHIA SINUATA V TABELLARIA	1	-	1	1	-	-	1
NITZSCHIA SP 6	-	-	-	-	-	-	-
STEPHANODISCUS ASTRAEA	-	-	-	-	-	-	-
STEPHANODISCUS ASTRAEA V. MINUTULA	1	-	2	4	18	11	38
STEPHANODISCUS INVISITATUS	-	-	5	30	6	2	2
SURIRELLA OVATA	-	-	-	-	-	-	-
SURIRELLA SUECICA	-	-	-	10	-	-	1
SYNEDRA DELICATISSIMA	-	-	-	10	-	-	-
SYNEDRA RUMPENS	19	-	12	35	8	1	56
SYNEDRA RUMPENS V FAMILIARIS	-	-	-	-	-	-	-
SYNEDRA ULNA	-	-	1	-	-	-	-
TABELLARIA FLOCCULOSA	-	-	-	-	-	-	-
TOTAL NUMBER OF ORGANISMS	494	507	491	454	495	484	462
NUMBER OF TAXA	19	9	37	46	36	42	43

TABLE 10. (Continued)

Taxonomic Classification	Number of Organisms at station:						
	6BB	6CM	7AB	7BB	7CM	8AB	8BM
BACILLARIOPHYTA (DIATOMS)							
ACHNANTHES EXIGUA V HETEROVALVE	32	1	2	2	-	-	-
ACHNANTHES LANCEOLATA V DUBIA	-	-	-	1	2	1	-
ACHNANTHES MINUTISSIMA	131	103	190	104	22	340	326
ACHNANTHES NULLII	86	10	76	42	2	8	14
ANOMOEONEIS VITREA	2	1	1	2	1	8	26
ASTERIONELLA FORMOSA	-	5	1	-	1	-	1
CALONEIS HYALINA	-	-	-	-	-	-	-
COCCONEIS PLACENTULA V EUGLYPTA	-	-	-	-	-	-	-
CYCLOTELLA ATOMUS	-	3	-	-	-	-	-
CYCLOTELLA MENEGHINIANA	1	-	-	-	-	-	-
CYCLOTELLA STELLIGERA	1	-	16	7	1	1	-
CYMBELLA AFFINIS	1	-	-	-	-	-	2
CYMBELLA DELICATULA	24	-	-	-	-	-	-
CYMBELLA LAEVIS	4	1	2	5	-	6	4
CYMBELLA MICROCEPHALA	4	-	2	5	-	6	1
CYMBELLA PROSTRATA V AUERSWALDII	13	15	5	17	18	8	4
CYMBELLA TUMIDA	-	-	-	1	-	8	-
DIATOMA TENUE V ELONGATUM	3	7	12	8	10	4	16
EUNOTIA PECTINALIS V MINOR	-	-	-	-	-	-	-
FRAGILARIA CAPUCINA V MESOLEPTA	1	1	-	-	2	-	1
FRAGILARIA CAPUCINA	-	17	13	16	223	17	16
FRAGILARIA CONSTRUENS N	-	-	-	-	-	-	-
FRAGILARIA CONSTRUENS V VENTER	-	-	-	-	-	1	-
FRAGILARIA PINNATA	-	-	-	-	-	-	-
FRAGILARIA VAUCHERIAE	15	7	27	76	27	10	32
GOMPHONEMA ANGUSTATUM	-	-	-	-	2	-	-
GOMPHONEMA PARVULUM	1	-	-	1	2	-	2
MELOSIRA DISTANS	2	7	1	-	2	1	-
MELOSIRA GRANULATA	3	14	4	5	4	2	1
MELOSIRA ITALICA	3	19	-	4	-	-	-
MELOSIRA VARIANS	-	1	2	1	1	2	2
NAVICULA CAPITATA V HUNGARICA	-	6	-	-	1	-	-
NAVICULA CINCTA	17	-	14	22	1	4	-
NAVICULA CONFERACEA V PENEGRINA	-	-	-	-	-	-	1
NAVICULA CONTENTA V BICEPS	-	-	-	-	-	-	-
NAVICULA CRYPTOCEPHALA	6	11	-	9	32	-	1
NAVICULA MENISCULUS V UPSALIENSIS	1	6	1	2	5	-	-
NAVICULA MINIMA	10	-	1	5	-	5	-
NAVICULA MUTICA	-	-	-	-	-	-	-
NAVICULA PELLICULOSA	-	1	-	-	-	-	-
NAVICULA PUPULA V MUTATA	-	1	-	-	3	-	-
NAVICULA RADIOSA N	1	-	2	-	-	-	-
NAVICULA RADIOSA V TENELLA	67	2	23	35	1	6	-
NAVICULA RHYNCOCEPHALA V GERMANI	-	-	-	-	1	6	-
NAVICULA SALINARUM V INTERMEDIA	-	-	-	-	-	-	-
NAVICULA SECRETA V APICULATA	4	18	-	9	11	-	-
NAVICULA SEMINULUM	-	-	-	-	-	-	-
NAVICULA CF SIMOLA	-	-	-	-	-	-	-
NAVICULA SUBHAMULATA	-	-	-	-	1	-	-
NAVICULA SUBTILISSIMA	-	1	-	1	1	2	-
NAVICULA THIENEMANNII	3	4	-	-	-	2	-
NAVICULA SP 6	-	-	-	-	-	-	-
NITZSCHIA ACCOMODATA	-	-	-	-	1	-	-
NITZSCHIA ACCUMUDATA	-	-	-	-	-	-	-
NITZSCHIA AMPHIBIA	-	-	-	-	-	-	-
NITZSCHIA BITA	-	-	-	-	-	-	-
NITZSCHIA DISSIPATA	-	22	-	1	5	1	1
NITZSCHIA DENTICULA	-	-	-	-	-	-	-
NITZSCHIA FILIFORMIS	-	-	1	-	1	-	-
NITZSCHIA FAUSTULUM V SUBSALINA	6	11	2	8	2	1	-
NITZSCHIA KUETZINGIANA	3	15	1	20	16	20	1
NITZSCHIA PALEA	1	18	-	20	24	20	-
NITZSCHIA RECTA	-	-	-	-	-	-	-
NITZSCHIA SINUATA V TABELLARIA	2	10	-	3	1	3	-
NITZSCHIA SP B	-	2	-	-	-	-	-
STEPHANODISCUS ASTRAEA	-	-	1	-	-	-	-
STEPHANODISCUS ASTRAEA V. MINUTULA	6	5	16	7	24	16	6
STEPHANODISCUS INVISITATUS	18	37	18	11	4	14	17
SURIRELLA OVATA	-	6	-	-	-	-	1
SURIRELLA SUECICA	1	-	-	-	1	-	-
SYNEDRA DELICATISSIMA	-	3	-	-	-	-	-
SYNEDRA RUMPENS	19	69	36	63	37	21	17
SYNEDRA RUMPENS V FAMILIARIS	4	-	1	1	3	1	1
SYNEDRA ULNA	1	-	-	-	-	-	-
TABELLARIA FLOCCULOSA	-	-	-	-	-	-	-
TOTAL NUMBER OF ORGANISMS	502	492	496	496	501	497	499
NUMBER OF TAXA	38	40	30	40	46	32	26

TABLE 10.(Continued)

Taxonomic Classification	Number of Organisms at station:						
	9AM	9BR	9CR	10AB	10BR	10CR	11AB
BACILLARIOPHYTA (DIATOMS)							
ACHNANTHES EXIGUA V HETEROVALVE	-	1	2	-	4	1	-
ACHNANTHES LANCEOLATA V DUBIA	1	3	6	1	7	8	-
ACHNANTHES MINUTISSIMA	206	41	47	8	61	91	253
ACHNANTHES NULLII	16	13	9	5	27	45	11
ANOMOEONEIS VITREA	5	-	-	2	3	-	4
ASTERIONELLA FORMOSA	-	2	1	6	-	-	1
CALONEIS HYALINA	-	-	-	-	-	-	-
COCCONEIS PLACENTULA V EUGLYPTA	-	1	-	-	1	2	1
CYCLOTELLA ATOMUS	-	-	-	-	-	-	-
CYCLOTELLA MENEGHINIANA	-	-	-	-	-	-	-
CYCLOTELLA STELLIGERA	-	-	-	-	-	-	-
CYMBELLA AFFINIS	20	-	8	-	1	5	-
CYMBELLA DELICATULA	1	-	-	-	2	-	11
CYMBELLA LAEVIS	7	2	-	8	2	3	3
CYMBELLA MICROCEPHALA	5	-	7	1	2	2	8
CYMBELLA PROSTRATA V AUERSWALDII	26	10	9	104	40	10	10
CYMBELLA TUMIDA	1	-	-	1	-	-	-
DIATOMA TENUE V ELONGATUM	29	23	22	55	19	12	7
EUNOTIA PECTINALIS V MINOR	-	-	-	-	-	-	-
FRAGILARIA CAPUCINA V MESOLEPTA	1	-	8	4	-	3	-
FRAGILARIA CAPUCINA	27	31	108	70	6	18	19
FRAGILARIA CONSTRUENS N	1	-	15	-	-	-	-
FRAGILARIA CONSTRUENS V VENTER	-	-	14	-	2	-	-
FRAGILARIA PINNATA	1	-	7	-	-	-	-
FRAGILARIA VAUCHERIAE	32	79	26	168	77	31	10
GOMPHONEMA ANGUSTATUM	-	-	1	-	2	-	-
GOMPHONEMA PARVULUM	-	-	6	-	-	-	7
MELOSIRA DISTANS	2	2	3	6	2	2	3
MELOSIRA GRANULATA	2	10	1	6	6	6	11
MELOSIRA ITALICA	-	2	-	-	5	4	1
MELOSIRA VARIANS	5	7	3	5	12	3	1
NAVICULA CAPITATA V HUNGARICA	2	2	4	2	3	2	-
NAVICULA CINCTA	2	2	2	-	-	-	20
NAVICULA CONFERACEA V PEREGRINA	-	-	1	-	-	-	-
NAVICULA CONTENTA V BICEPS	-	-	-	-	-	-	-
NAVICULA CRYPTOCEPHALA	-	10	15	3	5	11	1
NAVICULA MENISCULUS V UPSALIENSIS	3	1	3	1	10	1	1
NAVICULA MINIMA	-	7	8	1	14	15	1
NAVICULA MUTICA	-	-	-	-	-	-	-
NAVICULA PELLICULOSA	-	-	1	-	-	-	-
NAVICULA PUPULA V MUTATA	-	-	-	-	1	1	-
NAVICULA RADIOSA N	-	-	-	-	1	-	1
NAVICULA RADIOSA V TENELLA	-	1	-	1	5	1	8
NAVICULA RHYNCOCEPHALA V GERMANI	-	1	-	-	-	-	-
NAVICULA SALINARUM V INTERMEDIA	-	6	4	3	12	9	-
NAVICULA SECRETA V APICULATA	-	3	5	1	9	8	6
NAVICULA SEMINULUM	-	-	-	-	2	-	-
NAVICULA CF SIMULA	-	-	-	-	-	-	6
NAVICULA SUBHAMULATA	-	-	-	-	-	-	-
NAVICULA SUBTILISSIMA	-	1	-	-	1	2	5
NAVICULA THIENEMANNII	-	-	-	1	-	-	1
NAVICULA SP A	-	-	-	-	-	-	-
NITZSCHIA ACCOMODATA	-	-	-	-	-	-	-
NITZSCHIA ACCOMODATA	-	-	-	-	-	-	-
NITZSCHIA AMPHIBIA	-	-	-	-	1	-	-
NITZSCHIA CITA	-	-	-	-	-	1	-
NITZSCHIA DISSIPATA	6	67	12	6	118	32	3
NITZSCHIA DENTICULA	-	-	5	-	2	-	-
NITZSCHIA FILIFORMIS	-	1	1	-	1	1	8
NITZSCHIA PAUSTULUM V SUBSALINA	1	1	1	-	2	-	3
NITZSCHIA KUETZINGIANA	-	4	9	-	2	-	-
NITZSCHIA MALEA	-	2	1	-	-	6	1
NITZSCHIA RECTA	-	5	-	-	-	-	-
NITZSCHIA SINUATA V TABELLARIA	1	4	6	2	1	12	13
NITZSCHIA SP 6	-	-	-	-	-	2	1
STEPHANODISCUS ASTRAEA	-	-	1	-	12	2	1
STEPHANODISCUS ASTRAEA V. MINUTULA	32	23	36	26	20	27	19
STEPHANODISCUS INVISITATUS	70	76	64	28	46	64	14
SURIRELLA OVATA	1	-	5	-	2	1	-
SURIRELLA SUECICA	-	1	2	8	8	3	3
SYNEDRA DELICATISSIMA	1	-	1	-	-	-	-
SYNEDRA RUMPENS	13	25	1	17	8	28	4
SYNEDRA RUMPENS V FAMILIARIS	-	8	-	2	1	2	-
SYNEDRA ULNA	-	1	-	2	-	2	-
TABELLARIA FLOCCULOSA	-	-	-	-	-	-	-
TOTAL NUMBER OF ORGANISMS	514	480	496	518	569	489	486
NUMBER OF TAXA	28	38	44	30	46	43	41

TABLE 10 (Continued)

Taxonomic Classification	Number of Organisms at station: 11BW	11CW	12AR	12BB	12CW
BACILLARIOPHYTA (DIATOMS)					
ACHNANTHES EXIGUA V HETEROVALVE	1	2	1	18	2
ACHNANTHES LANCEOLATA V DUBIA	1	2		2	
ACHNANTHES MINUTISSIMA	22	46	46	69	56
ACHNANTHES NULLII	22	24	1	36	1
ANDONEONEIS VITREA	2	1	2	2	1
ASTERIONELLA FORMOSA		1		1	
CALONEIS HYALINA			1	1	1
COCCONEIS PLACENTULA V EUGLYPTA					
CYCLOTELLA ATOMUS					
CYCLOTELLA MENEGHINIANA					
CYCLOTELLA STELLIGERA					
CYMBELLA AFFINIS	1	1		1	
CYMBELLA DELICATULA	17	1		96	
CYMBELLA LAEVIS	3	1		2	
CYMBELLA MICROCEPHALA		16		10	
CYMBELLA PROSTRATA V AUERSWALDII	13	3	1	20	17
CYMBELLA TUMIDA				4	
DIATOMA TENUE V ELONGATUM	33	4	11	4	17
EUNOTIA PECTINALIS V MINOR					
FRAGILARIA CAPUCINA V MESOLEPTA					
FRAGILARIA CAPUCINA	241	6	31	9	126
FRAGILARIA CONSTRUENS N					
FRAGILARIA CONSTRUENS V VENTER					
FRAGILARIA PINNATA					
FRAGILARIA VAUCHERIAE	51	16	17	18	10
GOMPHONEMA ANGUSTATUM	1	2	1	1	27
GOMPHONEMA PARVULUM	5	2	7	4	9
MELOSIRA DISTANS	1	5	2		3
MELOSIRA GRANULATA	12	45	25	5	11
MELOSIRA ITALICA		5			2
MELOSIRA VARIANS	5	2	1	6	2
NAVICULA CAPITATA V HUNGARICA	5	2	1	28	3
NAVICULA CINCTA					
NAVICULA CONFERACEA V PEREGRINA		1			
NAVICULA CONTENTA V BICEPS			5	1	
NAVICULA CRYPTOCEPHALA		7	5		19
NAVICULA MENISCULUS V UPSALIENSIS	1	4	1	4	4
NAVICULA MINIMA	1	11	19	8	1
NAVICULA MUTICA		6			
NAVICULA PELLICULOSA			1		
NAVICULA PUPULA V MUTATA		1			12
NAVICULA RADIOSA N		1		8	
NAVICULA RADIOSA V TENELLA	12	1	1	64	
NAVICULA RHYNCOCEPHALA V GENMANI	1	2	2	1	2
NAVICULA SALINARIUM V INTERMEDIA		3			
NAVICULA SECRETA V APICULATA		2	7	2	
NAVICULA SEMINULUM	1	2		6	8
NAVICULA CF SIMULA					
NAVICULA SUBAMULATA		1	1	1	
NAVICULA SUBTILISSIMA		4	24	10	2
NAVICULA THIENEMANNII					
NAVICULA SP 6					
NITZSCHIA ACCOMODATA		2		9	
NITZSCHIA ACCOMODATA					
NITZSCHIA AMPHIBIA		2	6	8	3
NITZSCHIA BITA	3	2	1	8	3
NITZSCHIA DISSIPATA		11			
NITZSCHIA DENTICULA		2	5	1	3
NITZSCHIA FILIFORMIS	1	2			
NITZSCHIA FRUSTULUM V SUBSALINA			5		
NITZSCHIA KUETZINGIANA		10	19	1	20
NITZSCHIA PALEA	1	3	2	1	1
NITZSCHIA RECTA		3			
NITZSCHIA SINUATA V TABELLARIA	1	2	1	3	1
NITZSCHIA SP 6	1	1	256	1	2
STEPHANODISCUS ASTRAEA					
STEPHANODISCUS ASTRAEA V. MINUTULA	2	22	64	10	16
STEPHANODISCUS INVISITATUS	2	3	5	17	3
SURIRELLA UVATA		1			
SURIRELLA SUECICA	1	2	6	2	2
SYNEDRA DELICATISSIMA	4	7	6	2	2
SYNEDRA RUMPENS		2		28	23
SYNEDRA RUMPENS V FAMILIARIS	3	1	21	3	1
SYNEDRA ULNA	6	1	6	1	5
TABELLARIA FLOCCULOSA					
TOTAL NUMBER OF ORGANISMS	499	499	478	520	501
NUMBER OF TAXA	35	51	46	41	46

TABLE 10. (Continued)

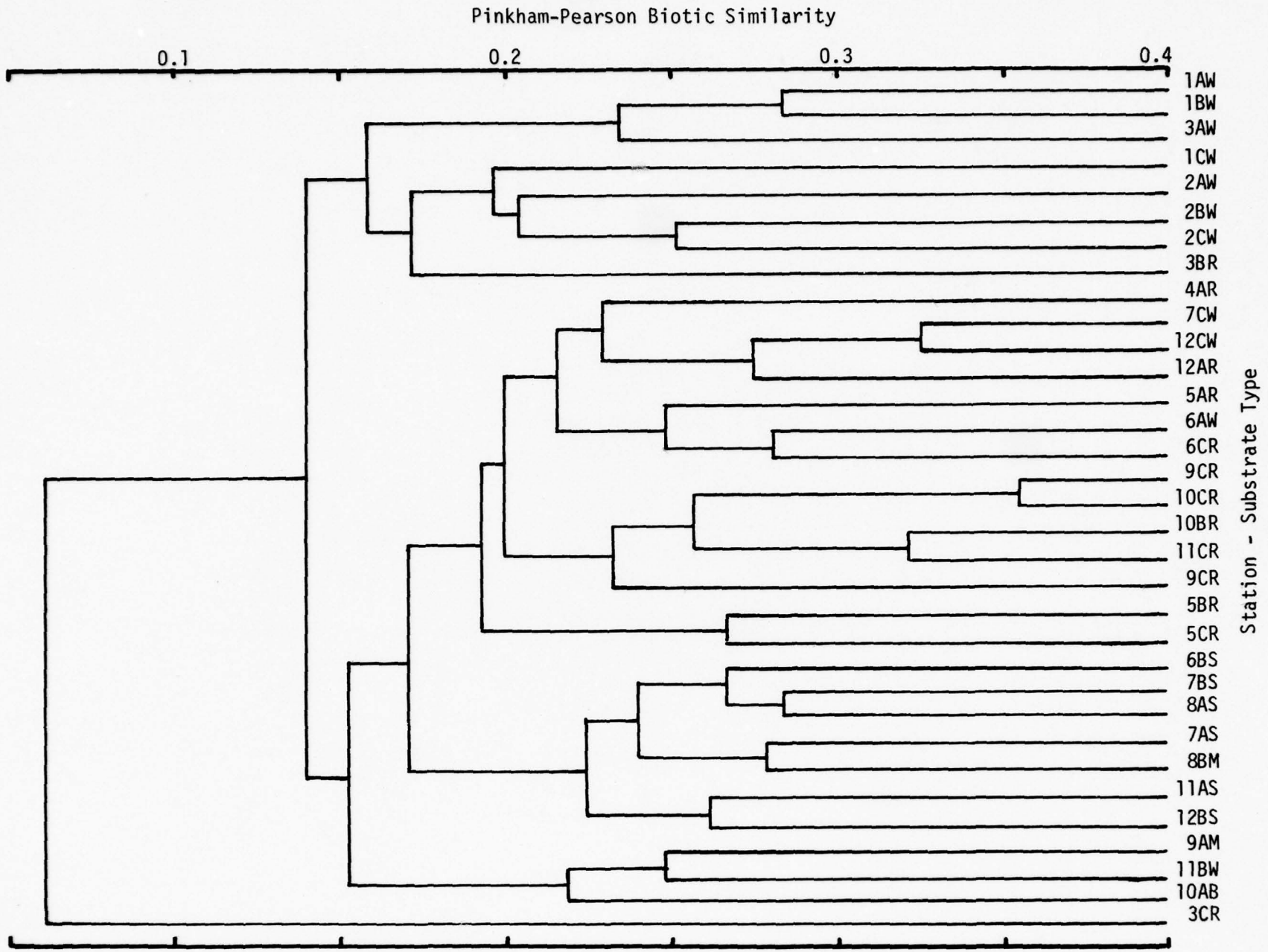

FIGURE 11. PHENOGRAM OF PERIPHYTON, NATURAL SUBSTRATE, UNCULLED, MUTUAL ABSENCE UNIMPORTANT, COPHENETIC CORRELATION COEFFICIENT 0.734.

TABLE 11

PRESENCE - ABSENCE DATA FOR FILAMENTOUS ORGANISMS
COLLECTED FROM VAAP ARTIFICIAL SUBSTRATES - MARCH, 1977

SPECIES	STATION NUMBER											
	1	2	3	4	5	6	7	8	9	10	11	12
Cyanophyceae												
Anabaena cf articulata												X
Oscillatoria sp 1		X		X		X	X	X	X		X	X
Schizothrix calcicola*	X	X	X	X	X	X	X	X	X		X	X
Chlorophyceae												
Mougeotia sp 1											X	X
Rhizoclonium sp 1											X	X
Stigeoclonium spl				X		X			X		X	X

Substrate Out of Water

* dominant at all stations

Three species of Chlorophyta were present. Of these, only Stigeoclonium occurred in the impact zone between stations 1 through 5. Green algae represented by Stigeoclonium, Rhizoclonium and Mougeotia were found more frequently in the reference bay. Only Stigeoclonium was present in Waconda Bay samples.

Chlorophyll a and Organic Biomass

Estimates of plant pigment and biomass levels were completed at all stations except S-10, where the periphytometer suspension unit was lost. Sixteen replicate slides for each of the two analyses were collected from the suspension units. These data are presented for each station in Figures 12 and 13. Table 12 contains summary information for means and ranges as well as the calculated Autotrophic Index based on mean values per station.

Chlorophyll a concentration on colonized slides at the various stations revealed a pattern consistent with population counts. Stations 1 through 5 had virtually no chlorophyll but a sharp increase occurred at S-6. A maximum average concentration of 40 mg/m^2 was observed at Station 7. Pigment levels gradually declined at downbay stations to 17 mg/m^2 at S-9. The reference bay samples showed means of 14 and 28 and could be considered comparable to outer Waconda Bay.

Organic biomass shows a similar pattern. Some heterotrophic growth occurs in the high stress zone between stations 1 through 5. Further downbay periphyton biomass increases with a maximum of 4.8 gms/m^2 at S-8. A maximum mean level of 6.2 mg/m^2 was observed at S-11 in the Reference Bay.

Calculation of the autotrophic index suggests an imbalanced environment among all stations except S-7. According to Weber (1973), ratios greater than 100 indicate organic pollution. Autotrophic index values for the winter survey ranged from 90 to 55,000. Maximum ratios were observed at stations 1 through 3.

Vital Stain

Three replicate slides from the periphytometer units were exposed to tetrazolium violet to estimate the physiological activity of diatoms. Cells were separated into live, senescent, or dead, based on the uptake of dye and the presence of chlorophyll. Cells completely filled with stain were classified as live. Those which contained chlorophyll but no stain were considered senescent. Those with neither dye nor chlorophyll were classified as dead. The technique offers some insight into growth characteristics of diatoms. Present methods of diatom enumeration using cleaned frustules probably leads to overestimates in that all cells are considered to be viable.

The data have been expressed as fractions using each category as it is related to the total number of individuals observed. This information as shown in Table 13 reveals that conditions existing in the channel between stations 1 and 4 resulted in nonviable populations that ranged up to 46 percent. A sharp demarcation occurred at station 5 where the percent dead organisms dropped to 7 percent. A further reduction to 1 percent was noted at station 6 where 93 percent of the organisms were vital staining.

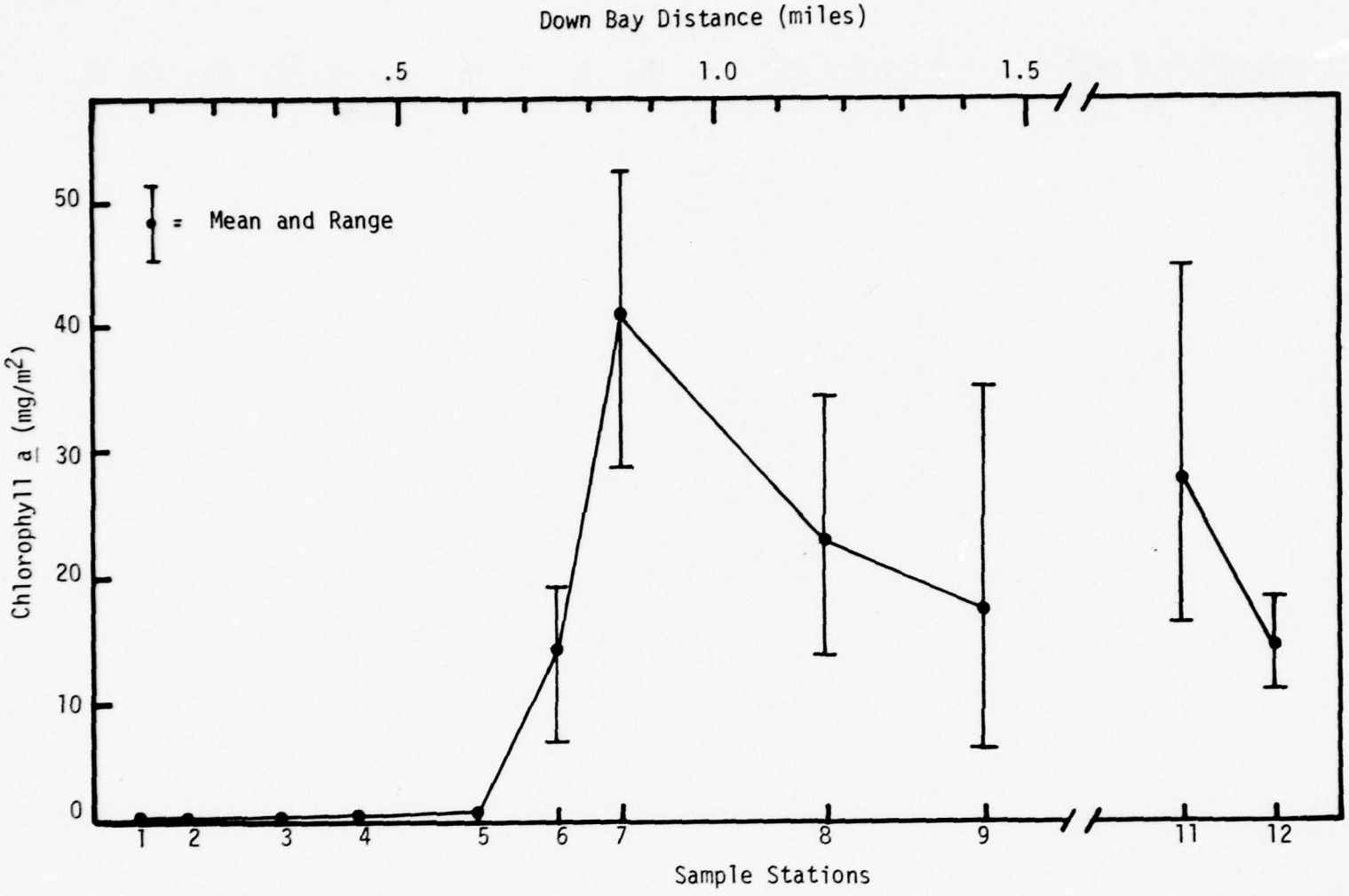

FIGURE 12. PERIPHYTON CHLOROPHYLL a, ARTIFICIAL SUBSTRATE, MARCH 1977

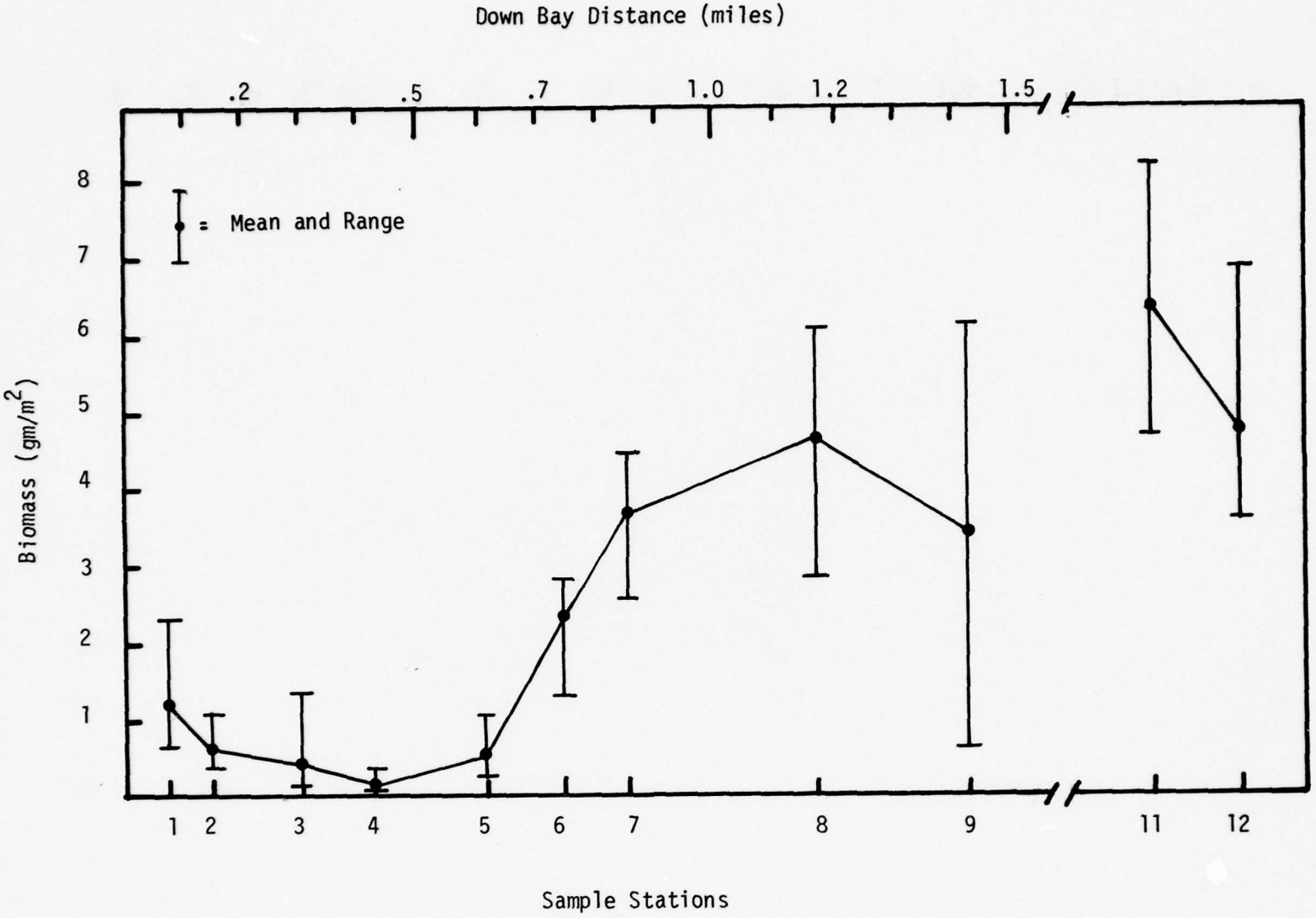

FIGURE 13. PERIPHYTON BIOMASS, ARTIFICIAL SUBSTRATE , MARCH 1977

TABLE 12

PERIPHYTON CHLOROPHYLL a, BIOMASS AND AUTOTROPHIC INDEX, MARCH 1977

Station	Chlorophyll a mg/m^2		Organic Biomass gm/m^2		Autotrophic Index
	Mean	Range	Mean	Range	
1	.02	.01-.06	1.1	.61-2.2	55,000
2	.02	.01-.04	.53	.27-1.0	26,500
3	.08	.02-.1	.34	.02-1.3	4,250
4	.2	.1 -.4	.10	.02- .26	500
5	.5	.2 -.8	.50	.22-1.0	1,000
6	14	7 - 19	2.3	1.2 -2.8	164
7	41	29 - 52	3.7	2.6 -4.4	90
8	23	13 - 34	4.8	2.9 -6.0	209
9	17	5 - 35	3.4	.57-6.1	200
10	-	-	-	-	-
11	28	16 - 44	6.2	4.7 -8.2	221
12	14	11 - 18	4.7	3.6 -6.8	336

TABLE 13

VIABLE CELL RATIOS IN VAAP PERIPHYTON, MARCH, 1977

	Live	Senescent	Dead
1	.25	.63	.13
2	.08	.46	.46
3	.48	.29	.24
4	.47	.20	.33
5	.48	.45	.07
6	.93	.06	.01
7	.89	.09	.01
8	.71	.25	.04
9	.72	.24	.03
10	---	---	---
11	.62	.27	.12
12	.70	.19	.11

PHASE II - MACROINVERTEBRATES

Introduction

Aquatic macroinvertebrates are a diverse group of small aquatic animals comprised of snails, clams, arthropods, annelids (segmented worms and leeches), planarians, and coelenterates. Of these, oligochaetes and chironomid (midge fly) larvae account for the majority of the organisms in this study.

Aquatic macroinvertebrates are a major biological component of aquatic systems and form an important part of the food chain. They feed on detritus and microscopic plants and animals. They, in turn, are eaten by small fish which support the larger and more economically and recreationally important species. Macroinvertebrates are of special importance in stream environments because of their role in recycling large amounts of organic detritus introduced from uplands.

Macroinvertebrates were selected as a parameter for this study because they are sensitive to environmental changes and thus, are important indicators of water quality. Natural or man-induced fluctuations in the physical-chemical characteristics of a lentic system are reflected by shifts in benthic community structure. They tend to remain at fixed locations and have a relatively short life span of usually a year or less and therefore, reflect both the present and recent past environmental conditions.

Macroinvertebrate species composition (population size, number of taxa, and diversity) is primarily dependent on three factors -- water quantity, water quality, and substrate composition.

Water quantity limits species within a site. For example, some organisms prefer large, deep lakes while others are found in small, shallow lakes. Water quality is also a significant factor in determining the assemblage of macroinvertebrates. Principal parameters include oxygen, temperature, hardness, related cations, and dissolved solids. The most important of these is oxygen. Increasing evidence shows that adverse effects can be expected on macroinvertebrates in environments supersaturated with oxygen as well as those that are undersaturated. Aquatic macroinvertebrates are also affected by temperature extremes. The Aquatic Life Advisory Committee (1956) indicates that benthic communities in temperature zones are adapted to seasonal fluctuations of temperature between 0 and 32°C (32 - 90°F).

Substrate is the most important determinant in species composition (Hynes, 1960). There is a direct relationship between the amount of available surface area and species abundance and diversity. That is to say, there are more hiding and foraging places in a rock or pebble bottom than in a sand or mud bottom. Contribution of organic matter, particularly from plants, is also an important consideration. Aquatic plants increase the abundance and diversity of benthic organisms viz. there is more surface area, periphytic food organisms, food from the plants themselves, and detritus from which to feed. Beck (1954) states, "...after careful examination of many streams, diversity of fauna was primarily the result of one factor -- the diversity of habitat."

Environmental Conditions

The winter of 1976-1977 was severe and unusually low temperatures were recorded throughout the season. Ice cover on Waconda Bay prevented the commencement of Phase II until late February. These conditions influenced the composition of the macroinvertebrate community by reducing populations. Low lake levels caused a significant reduction in water surface area in the upper end of Waconda Bay. Effluent from VAAP was confined to a relatively narrow, shallow channel for some 3,000 feet downbay after reaching reservoir pool elevation. Lack of mixing in this channel caused significant concentrations of effluent to extend further downbay than would be expected at higher pool elevations.

Detritus was present at all stations, but was abundant only at Station 6. A mat of filamentous algae grading from heavy to sparse between stations 3-6 was also observed. The slender spikerush (Eleocharis acicularis) was found in small quantities at Station 12.

Table 14 presents the characteristics of the bay sediments at the various stations. Except for Stations 8, 9, and 10, all samples were obtained at comparable depths. During Phase II, 15 replicate grabs were collected at each station. In addition, 14 Hester-Dendy 5-plate units were placed at each station location for colonization over a 4-week period.

Natural Substrate Colonization

The results for this part of the study are shown in Table 15 and are expressed as numbers of organisms/square meter. The various taxa are grouped into two categories representing chironomids and other forms. Except at Station 6, midges composed 50 percent or more of the total population. Three forms were most abundant. These were Procladius sp, Coelotanypus spp. and Chironomus sp. Procladius sp. dominated at Stations 1-5 and may represent forms more tolerant to VAAP waste.

Total population/m^2 ranged from 586 to 1,318. Numbers of species were generally uniformly distributed with 9 stations showing 10 - 15 species each. The lowest number of taxa (7) was noted at Station 2.

Other forms such as oligochaetes were not abundant. The largest population amounted to 793 organisms/m^2 at Station 6. Populations of fingernail clams were rather sparse between Stations 1 and 4. Further downbay where effluent effects would be less, these fingernail clams were observed ranging from 60 to 190/m^2. Other organisms demonstrated similar patterns. Hexagenia mayflies were found at Station 3 indicating, at least, some tolerance to the waste in this location. A large increase in these insects was observed at Stations 4, 5, and 6. Shannon-Weaver Diversity is expressed both for culled and unculled data in Table 16. For natural substrates values range to slightly above 2 for each of the culling routines. Station 3 represents the area of highest diversity followed by Station 8. Stations 1 and 2 in the maximum zone of impact were the least diverse. In general, there seemed to be no relationship between diversity and station location vis-a-vis the waste outfall.

TABLE 14

CHARACTERISTICS OF THE BENTHIC SUBSTRATE AT THE SAMPLING STATIONS WITHIN WACONDA BAY AND THE REFERENCE BAY, AS OBSERVED FROM DREDGED MACROINVERTEBRATE SAMPLES.

Substrate Characteristics	Stations											
	1	2	3	4	5	6	7	8	9	10	11	12
Depth (meters)	1.5	1.5	1.5	1.5	1.5	1.3	2	3	4	4	1.5	.75
Silt & clay*												
(a) orange	1-2	1-2	2-4	2-4	2-4	V						P
(b) gray	P	P	P	P	P	P	P	P	P	P	P	
(c) brown							P	P	P	P	P	P
(d) oil						P						
Gravel									P			
Detritus	P	P	P	P	P	A	P	P	P	P	P	P
Fil. Algae			P	P	P	V						
Eleocharis												P
Hexagenia burrows							A	A	A	P	P	
Decaying fish	A	A	A	P	P							

*Depth in centimeters
P = Present
A = Abundant
V = Veneer

TABLE 15

VAAP MACROINVERTEBRATES, NATURAL SUBSTRATE, MARCH 1977

Taxonomic Classification	Number of Organisms per m^2 at Station:											
	1	2	3	4	5	6	7	8	9	10	11	12
CHIRONOMIDAE												
ABLABESMYIA ANNULATA	-	-	17	26	66	9	9	17	9	17	3	9
CHIRONOMUS SP	3	17	43	34	49	129	43	34	26	190	226	95
COELOTANYPUS CONCINNUS	6	-	34	60	20	-	129	17	26	-	3	-
COELOTANYPUS SCAPULARIS	-	-	26	17	17	9	-	26	77	60	11	17
KIEFFERULUS TENUIS	-	-	34	-	26	17	77	215	267	310	184	43
CRYPTOCHIRONOMUS FULVUS	-	-	9	9	14	-	-	9	-	-	6	9
DICROTENDIPES LEUCOSCELIS	-	-	-	-	3	-	-	-	-	-	-	-
DICROTENDIPES NEOMODESTUS	-	-	-	-	-	-	-	-	-	-	-	9
PROCLADIUS SP	1011	509	172	302	365	103	86	121	26	17	60	319
TANYPUS NEOPUNCTIPENNIS	118	17	9	-	-	-	-	-	-	-	-	-
TANYTARSUS SP C	-	-	9	-	-	-	-	-	-	-	-	9
NON-CHIRONOMIDAE												
ASELLUS SP	3	-	-	-	-	-	-	-	-	-	-	-
CERATOPOGONIDAE (NO LARVAL KEY)	3	34	9	9	6	-	-	-	-	-	3	26
CHAOBORUS SP	6	9	43	121	43	43	60	138	138	26	6	-
CORBICULA MANILENSIS	-	17	60	17	46	43	-	9	17	-	14	9
ENALLAGMA SP	6	-	-	-	-	-	-	-	-	-	-	-
HEXAGENIA SP	-	-	26	147	316	112	52	69	121	78	210	102
NEMATODA	3	9	-	-	-	-	-	-	9	-	-	-
OLIGOCHAETA	132	-	52	43	9	795	-	9	9	34	3	129
SPHAERIUM SP	3	-	43	9	109	60	190	129	60	60	101	86
UNIDENTIFIED COLEOPTERA	3	-	-	-	-	-	-	-	-	-	-	-
UNIDENTIFIED DIPTERA	3	-	-	-	-	-	-	-	-	-	-	-
TOTAL NUMBER OF ORGANISMS	1300	612	566	794	1089	1518	646	793	785	792	828	863
NUMBER OF TAXA	13	7	15	12	14	10	8	12	12	9	13	13

Unculled Data

TABLE 16

SHANNON - WEAVER DIVERSITY INDICES ($\overline{H}$) FOR VAAP NATURAL SUBSTRATES, MACROINVERTEBRATES MARCH, 1977

Station	Magnitude Culled	Magnitude Unculled
1	0.73	0.82
2	0.67	0.74
3	2.26	2.35
4	1.84	1.88
5	1.84	1.90
6	1.43	1.43
7	1.87	1.87
8	1.98	2.02
9	1.92	1.96
10	1.74	1.74
11	1.73	1.76
12	1.83	1.95

Base e

The data as analyzed by cluster analysis are presented in Figures 14 and 15. The phenograms are representative of two options in the analysis procedure. In Figure 14, organisms which were present at 4 percent or less of the total population at any station were culled and no longer considered in the analysis procedure. Using the Pinkham-Pearson Similarity Index, the option of mutual absence important was employed. Figure 15 shows the clustering pattern based on all organisms that were collected and where mutual absence was considered unimportant. These two routines were run to examine the impact on station clustering of organisms occurring on a seldom or rare basis. As the figures show, the clustering is relatively similar. In both cases stations 1 and 2 are highly unrelated to the remaining stations. Stations 3, 4, and 5 are somewhat similar. No really consistent pattern exists for the remaining stations indicating that stations 6 through 9 in Waconda Bay are not distinguishable from stations 10 through 12 in the reference bay.

Artificial Substrate Colonization

Overall, colonization of the Hester-Dendy units was sparse and densities were greatly reduced over those in the benthos. For example, chironomids were 2 to 3 orders of magnitude less than those populating sediments in Waconda Bay. Combined data from five Hester-Dendy units per station are presented in Table 17.

Chironomids dominated artificial substrates at Stations 7 through 9 in Waconda Bay, and Stations 10 and 11 in the reference bay. Cricotopus spp. accounted for about two-thirds of the Chironomidae. Several species of Cricotopus are involved; however, presently there are no effective means to separate these species. Four species of Dicrotendipes and two species of Glyptotendipes comprised most of the remaining chironomids. These six feed upon planktonic materials. Cricotopus was present throughout Waconda Bay, and was abundant in the reference bay. Dicrotendipes and Glyptotendipes were relatively common in the reference bay and were frequently found in the open bay stations (6 through 9) of Waconda Bay. Two other genera, Ablabesmyia and Tanytarsus, were frequently found in the reference bay, but rarely found in Waconda Bay.

Of the non-chironomid fauna, only two of the nine found on artificial substrates were present in significant numbers. The larvae of Caenis, a small herbivorous mayfly, reached its greatest population at Station 6 where it formed 61 percent of the population. Densities fell steeply either upbay or downbay. It was not collected at Stations 1, 2, 9, and 10. Oligochaetes occurred sporadically in Waconda Bay, but were the dominant taxa on reference bay artificial substrates. The larvae of Argia sp., a predaceous damselfly, were found only at Stations 1 and 2. This genus has previously been reported only from unpolluted situations (Beck, 1954) in Florida, but Beck (personal communication) believes this may not hold true for some non-Florida species.

The highest densities and numbers of species occurred in the reference bay stations which suggests some inhibition in all Waconda Bay samples. Considering total number of species per station the stations seem to group as follows: stations 1 to 5 averaged 3.3 species; stations 6 to 9 averaged 9.5

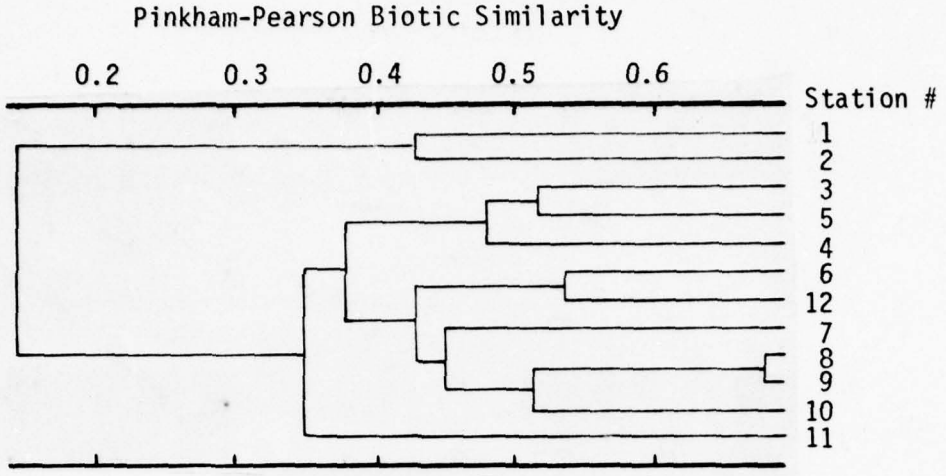

FIGURE 14 . PHENOGRAM OF MACROINVERTEBRATES, NATURAL SUBSTRATE, CULLED AT 4%, MUTUAL ABSENCE IMPORTANT. COPHENETIC CORRELATION COEFFICIENT, 0.917.

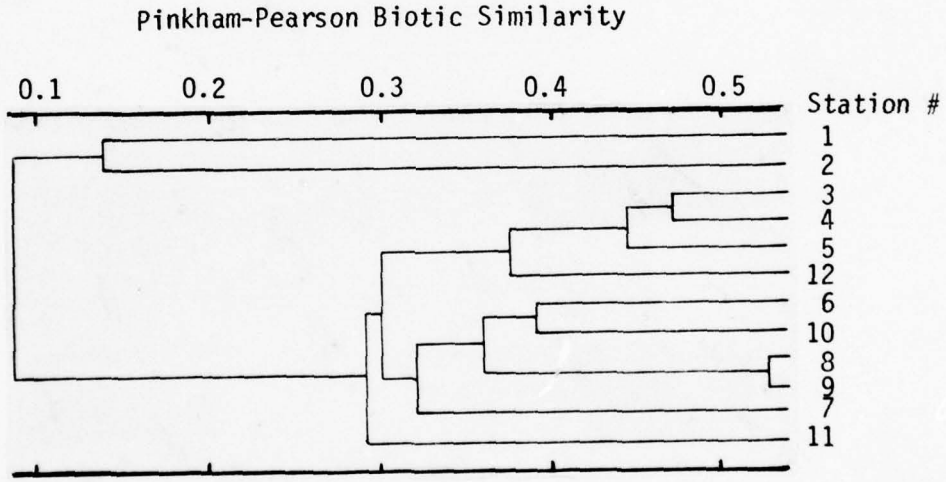

FIGURE 15 . PHENOGRAM OF MACROINVERTEBRATES, NATURAL SUBSTRATE, UNCULLED, MUTUAL ABSENCE UNIMPORTANT. COPHENETIC CORRELATION COEFFICIENT, 0.900.

TABLE 17

VAAP MACROINVERTEBRATES, ARTIFICIAL SUBSTRATE, UNCULLED, MARCH 1977

Taxonomic Classification	Number of Organisms per m^2 at Station: 1	2	3	4	5	6	7	8	9	10	11	12
CHIRONOMIDAE												
ABLABESMYIA MALLOCHI	-	-	-	-	-	7	-	-	3	10	20	3
ABLABESMYIA PARAJANTA	-	-	-	-	-	7	-	-	-	13	20	10
ABLABESMYIA TARELLA	-	-	-	-	-	-	-	-	-	-	3	3
CHIRONOMUS SP	-	-	-	-	-	-	-	10	-	-	3	3
CONCHAPELOPIA SP	-	-	-	-	-	-	-	-	-	-	-	-
CRICOTOPUS SP	3	-	3	-	3	10	20	23	17	90	210	1227
CRYPTOCHIRONOMUS FULVUS	-	-	-	-	-	-	-	-	-	-	-	-
DICROTENDIPES LEUCOSCELIS	-	-	-	-	-	-	3	7	-	7	3	7
DICROTENDIPES MODESTUS	-	-	-	-	3	17	3	-	-	10	37	120
DICROTENDIPES NEOMODESTUS	-	-	-	-	-	3	3	7	-	13	30	30
DICROTENDIPES NERVOSUS	-	-	-	-	-	-	-	10	-	7	10	13
GAMMARUS SP	-	-	-	-	-	-	-	-	-	-	-	20
GLYPTOTENDIPES LOBIFERUS	-	-	-	-	-	3	3	7	13	20	37	40
GLYPTOTENDIPES SP	-	-	-	-	-	-	-	-	-	3	-	13
MICROPSECTRA SP A	-	-	-	-	-	-	-	-	3	3	-	-
PARATANYTARSUS SP A	-	-	-	-	-	-	-	-	-	-	-	3
POLYPEDILUM FALLAX	-	-	-	-	-	-	-	-	3	3	-	-
POLYPEDILUM NEAR ILLINOENSE TYPE B	-	-	7	3	-	3	-	-	-	-	3	3
PSECTROCLADIUS SP A	-	-	-	-	-	-	-	7	-	-	-	-
TANYTARSUS SP C	-	-	-	-	-	3	3	-	-	33	33	37
THIENEMANNIMYIA SP	-	-	-	-	-	3	-	-	7	-	3	3
THISSOCLADIUS SP	-	-	-	-	-	-	-	-	-	-	-	-
UNIDENTIFIED ORTHOCLADIAN	-	-	-	-	-	-	-	-	3	-	-	-
NON-CHIRONOMIDAE												
ARGIA SP	3	10	-	-	-	-	-	3	-	-	-	7
CAENIS SP	-	-	13	50	66	106	50	3	-	3	13	30
CERATOPOGONIDAE (NO LARVAL KEY)	-	-	-	3	-	-	-	-	-	-	-	-
CORBICULA MANILENSIS	-	-	-	-	-	5	-	-	-	-	-	-
HAPLOTAXIDA	-	-	-	-	-	3	-	-	-	-	-	-
PARAPELLUS SP	-	-	-	7	-	-	-	-	-	-	-	-
PHYSELLA SP	-	3	-	-	-	-	-	-	-	-	-	3
PISIDIUM SP	-	-	-	-	-	-	-	-	-	-	-	3
ENALLAGMA SP	-	-	-	-	-	-	-	-	-	-	-	-
HYALELLA AZTECA	-	-	-	-	-	-	-	-	-	-	-	3
OLIGOCHAETA	-	-	-	-	-	7	3	-	20	157	287	1690
STENONEMA SP	-	-	-	-	-	-	-	-	-	-	3	-
TOTAL NUMBER OF ORGANISMS	6	13	23	69	69	175	68	77	69	372	718	3271
NUMBER OF TAXA	2	2	3	6	3	13	8	9	8	14	17	22

species; stations 10 and 11 averaged 15.5 species. Station 12 was excluded because the sampling rack had been overturned in the field resulting in the Hester-Dendy units being suspended at about one half the depth of the units at other stations.

Using the five replicates from each station, an analysis of variance was performed on the organism density data. These data were first transformed to ln (N + 1). The calculated F value (degrees of Freedom 11, 48) was 47.3 which is significant at less than 0.0001. Using Duncan's multiple range test (5% criteria) to compare treatment means it was found that significant differences in population density are as follows:

$$1, 2, 3 < 7, 5, 4, 9, 8 < 6 < 10 < 11 < 12$$

Table 18 shows diversity values for the community colonizing the Hester-Dendy units. The data are expressed using the culled-unculled format. Treatment of the data by either option shows Station 8 to be the most diverse with a maximum of 2.02. The lowest diversities were observed between Stations 1 through 5, suggesting that these stations were under greater stress.

Cluster analysis using the Pinkham-Pearson approach was performed on data from the Hester-Dendy units (Figures 16&17). The unculled data were analyzed on a mutual absence unimportant basis whereas when the data were culled, mutual absence was considered important. In the culled data, Stations 1 to 5 group separately as do Stations 11 and 12. In the unculled data, at Stations 1, 3, 4 and 5 are grouped together, Station 2 is unlike any other station and Stations 11 and 12 are highly similar. Thus, in both cases, Stations 1 to 5 seem to separate from the remaining group.

TABLE 18

SHANNON - WEAVER DIVERSITY INDICES ($\overline{H}$) FOR
VAAP ARTIFICIAL SUBSTRATES MACROINVERTEBRATES
MARCH, 1977

Station	Magnitude Culled	Magnitude Unculled
1	0.69	0.69
2	0.54	0.54
3	0.95	0.95
4	1.01	1.01
5	0.36	0.36
6	1.39	1.57
7	1.55	1.55
8	2.02	2.02
9	1.80	1.80
10	1.69	1.81
11	1.66	1.80
12	1.11	1.20

Base e

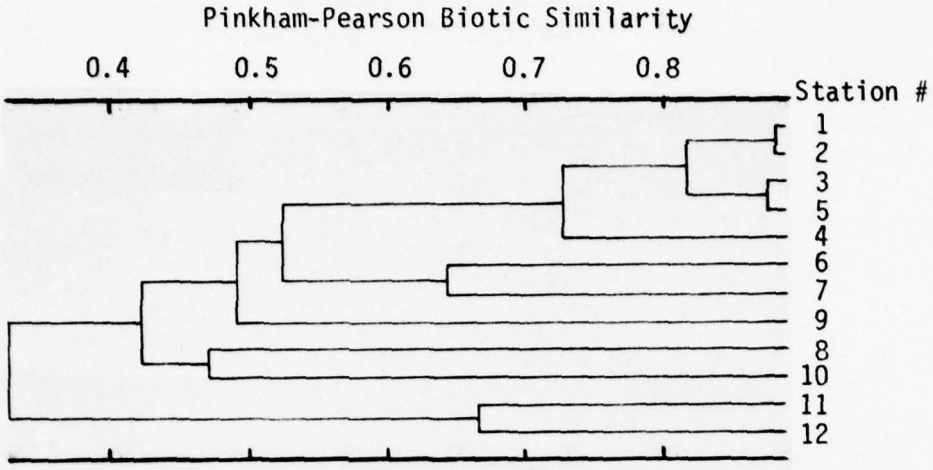

FIGURE 16 . PHENOGRAM OF MACROINVERTEBRATES, ARTIFICIAL SUBSTRATE, CULLED AT 4%, MUTUAL ABSENCE IMPORTANT. COPHENETIC CORRELATION COEFFICIENT, 0.902

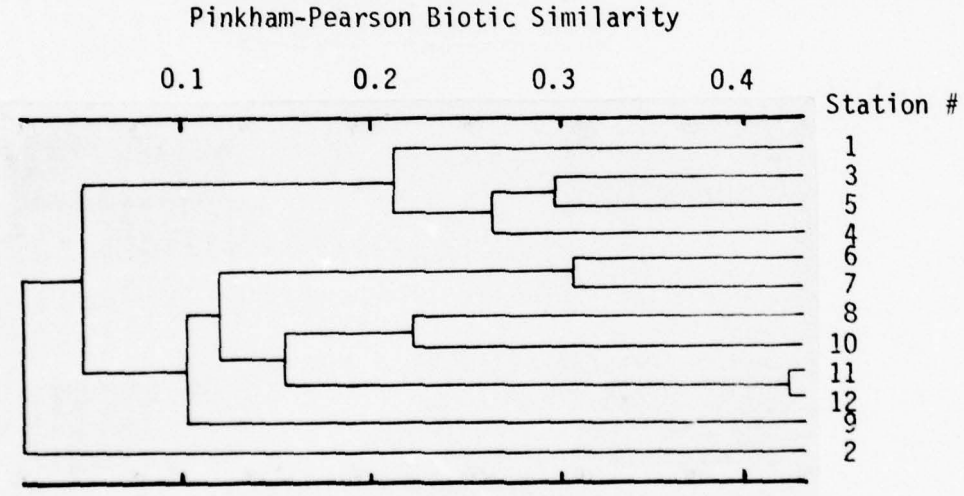

FIGURE 17 . PHENOGRAM OF MACROINVERTEBRATES, ARTIFICIAL SUBSTRATE, UNCULLED, MUTUAL ABSENCE UNIMPORTANT. COPHENETIC CORRELATION COEFFICIENT, 0.882.

DISCUSSION AND CONCLUSION

In relating environmental effects to munitions concentrations in a field study such as this, one should understand the several assumptions and/or limitations that are inherent. First, the concentrations at any site vary with time. For the natural substrate sampling, the concentrations measured from March 1-5 are presumed to be "normal" or "average" and the biologic communities are assumed to be in equilibrium with these concentrations. For the artificial substrates, measurements were made of munitions in the effluent during the incubation period but no bay samples were taken. However, from the effluent concentrations and an understanding of how the effluent mixes with bay waters, one can predict possible resultant bay concentrations. Also, the plant effluent contains a complex mixture of munitions-related organic compounds plus significant concentrations of other ions, particularly nitrate and sulfate. Thus the observed effects may not be due to only the five specific munitions compounds measured. Even with these limitations, certain conclusions can be relatively firm. Other conclusions can only be tentative due to the factors noted above and because the effects indicated are subtle.

Overall, the data appear to be very consistent. The water chemistry, artificial substrate periphyton population density, chlorophyll a, biomass and viable cell ratios, and artificial and natural substrate macroinvertebrate community structure all indicate that Stations 1-5 are affected by the VAAP discharge. The only data that do not support this conclusion are sediment chemistry and natural substrate periphyton. Given that the effluent does appear to be having an effect, the problem thus becomes one of relating the effects to specific causes.

First, at what concentration levels were no measurable effects observed? In the natural substrate no-effect could be discerned in either number of species, density, or community structure for both benthic and periphyton organisms at stations 6 through 9 as compared to reference bay stations 10 through 12. During the sampling period total concentration of the five munitions compounds averaged less than 25 µg/l at stations 6 through 9. In the December sampling average munitions levels in this area ranged as high as 27 µg/l. Hence, it appears that for total munitions concentration less than 25 µg/l no effects would be expected.

At the opposite end of this range, effluent munitions concentrations during the incubation period for the artificial substrate samples averaged about 575 µg/l and reached a peak of 2370 µg/l. Concentrations at stations 1 through 5 should not have exceeded these values. At this level, definite effects were noted in periphyton population density and community structure and in macroinvertebrate numbers of species and community structure. It cannot be determined whether the peak or average concentration was most influential in the result.

Between these extremes, effects were subtle and less pronounced. In the five day period prior to the incubation of the artificial substrate, munitions concentrations in the upper channel, stations 1 through 5, were

averaging about 80 µg/l. Data from the December survey and results of prior studies suggest that the long term average munitions concentration in this area might actually be somewhat higher. Even so, the only observed effect on macroinvertebrate infauna was a slight shift in community structure. Substrate variation in periphyton samples may have masked slight shifts in community structure in that compartment also.

During the incubation of the artificial substrate samples, it is predicted that the concentration of munitions compounds at stations 6 - 9 could have gone up to 50 - 100 µg/l. For macroinvertebrates, density, number of species, and community structure at stations 6 - 9 were different than at stations in the reference bay. For periphyton, the numbers of species seemed to be slightly lower at stations 6 to 9 as compared to the reference bay stations. These differences were not as substantial as those observed between the upbay and reference areas. However, they were of such magnitude as to eliminate them from the definite no-effect category.

In the 1975 study (Sullivan, et al., 1977), biostimulation was observed in certain portions of Waconda Bay. In this winter survey no such biostimulation was observed. Possibly the low temperature encountered prevented this.

In summary, the work reported herein indicates no environmental effects on periphyton or macroinvertebrates from a complex TNT manufacturing effluent at a total munitions concentration of less than 25 µg/l. Definite effects were noted during a period when total munitions averaged 500-600 µg/l with a peak of over 2000 µg/l. The data further suggests the tentative conclusion that concentrations in the range of 50-100 µg/l produce minimal effects. Again it should be noted that the concentrations of other non-munitions unique compounds changed as the munitions concentrations changed. These changes probably were factors also in the biologic changes observed.

These results are in good agreement with the conclusions of the 1975 study (Sullivan et al. 1977). In that work, toxicity was indicated in the range of 50-350 µg/l total munitions and no effects were observed at concentrations of 20 µg/l and less.

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APPENDIX A

PHASE I AND II SURVEY

Figures and Tables

LIST OF TABLES AND FIGURES
APPENDIX A

TABLE A-1

IN SITU MEASUREMENTS OF DISSOLVED
OXYGEN, TEMPERATURE AND CONDUCTIVITY
VAAP, DECEMBER 1976, PHASE I

Water and Air Research, Inc.
6821 S.W. Archer Road, P. O. Box 1121
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(904) 372-1500

JOB

SHEET NO. 1 OF 12

CALCULATED BY DPC DATE 21 DEC 76

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SCALE

STATION NO. A

DEPTH	PARAMETER	DATE 7 DEC	8 DEC	9 DEC	10 DEC	11 DEC	AVERAGE VALUE	RANGE
0.5 ft	TEMP, °C	9.0	8.0	7.0	6.0	7.0	7.4	6.0 - 9.0
	D.O., mg/l	11.4	11.7	11.6	11.5	11.2	11.5	11.2 - 11.7
	COND, μmho/cm	370	455	565	380	560	466	370 - 565
2.0 ft	TEMP, °C	9.0	8.0				8.5	8.0 - 9.0
	D.O., mg/l	10.9	11.3				11.1	10.9 - 11.3
	COND, μmho/cm	410	455				433	410 - 455
3.0 ft	TEMP, °C	9.0	8.0		6.0		7.7	6.0 - 9.0
	D.O., mg/l	10.8	11.1		11.5		11.1	10.8 - 11.5
	COND, μmho/cm	500	455		920		625	455 - 920
4.0 ft	TEMP, °C	9.0	~~3.5 ft~~ 8.0			9.5		
	D.O., mg/l	10.7	11.0			11.0		
	COND, μmho/cm	460	455			1130		
5.0 ft	TEMP, °C				8.0			
	D.O., mg/l				11.4			
	COND, μmho/cm				1700			
6.0 ft	TEMP, °C			7.0				
	D.O., mg/l			11.6				
	COND, μmho/cm			660				

* DOUBLE LINE INDICATES BOTTOM DEPTH

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SCALE

STATION NO. B-2

DEPTH	PARAMETER	DATE 7 DEC	8 DEC	9 DEC	10 DEC	11 DEC	AVERAGE VALUE	RANGE
0.5 ft	TEMP, °C	8.5	8.0	7.0	6.0	7.0	7.3	6.0 – 8.5
	D.O., mg/l	11.2	10.7	11.4	11.1	11.1	11.1	10.7 – 11.4
	COND, µmho/cm	500	440	510	490	480	484	440 – 510
2.0 ft	TEMP, °C	8.5	8.0				8.3	8.0 – 8.5
	D.O., mg/l	10.9	10.6				10.8	10.6 – 10.9
	COND, µmho/cm	520	440				480	440 – 520
3.0 ft	TEMP, °C	8.5	8.0			7.0	7.8	7.0 – 8.5
	D.O., mg/l	10.6	10.6			11.0	10.7	10.6 – 11.0
	COND, µmho/cm	550	440			605	532	440 – 605
4.0 ft	TEMP, °C	8.3	8.0		7.0		7.8	7.0 – 8.3
	D.O., mg/l	10.4	10.6		11.2		10.7	10.4 – 11.2
	COND, µmho/cm	645	460		1080		728	460 – 1080
5.0 ft	TEMP, °C	8.3	8.0				8.2	8.0 – 8.3
	D.O., mg/l	10.3	10.6				10.4	10.3 – 10.6
	COND, µmho/cm	655	600				628	600 – 655
5.5 ft	TEMP, °C ~~6.0 ft~~	8.3	8.0 ~~7 ft~~	6.5 ~~7 ft~~	8.3 ~~6 ft~~	9.0	DEPTH OF BOTTOM	
	D.O., mg/l	10.2	10.2	11.4	10.4	10.6		
	COND, µmho/cm	670	605	605	1900	1160		

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CALCULATED BY DPC DATE 21 DEC 76

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SCALE

STATION NO. C1

DEPTH	PARAMETER	DATE				
		7 DEC	8 DEC	9 DEC	10 DEC	11 DEC
0.5 ft	TEMP, °C		6.0	6.5		6.5
	D.O., mg/l		12.6	13.8		12.6
	COND, μmho/cm		200	160		180
1.0 ft	TEMP, °C	10.5			6.5	
	D.O., mg/l	8.0			11.0	
	COND, μmho/cm	400			390	

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CALCULATED BY DPC DATE 21 DEC 96
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SCALE

STATION NO. C2

DEPTH	PARAMETER	DATE 7 DEC	8 DEC	9 DEC	10 DEC	11 DEC	AVERAGE VALUE	RANGE
0.5 ft	TEMP, °C	8.5	6.5	6.5	5.5	6.5	6.7	5.5 - 8.5
	D.O., mg/l	11.3	12.6	12.2	13.2	12.3	12.3	11.3 - 13.2
	COND, µmho/cm	510	185	305	235	260	299	185 - 510
2.0 ft	TEMP, °C	8.5	6.5					
	D.O., mg/l	10.9	12.4					
	COND, µmho/cm	540	180					
3.0 ft	TEMP, °C	8.5						
	D.O., mg/l	10.7						
	COND, µmho/cm	545						
4.0 ft	TEMP, °C	8.5			5.8			
	D.O., mg/l	10.6			13.0			
	COND, µmho/cm	550			750			
5.0 ft	TEMP, °C	8.5	~~6.5 ft~~	6.5	~~5.5 ft~~ 7.5	no depth 7.5		DEPTH OF BOTTOM
	D.O., mg/l	10.5		11.4	10.4	10.4		
	COND, µmho/cm	550		400	~1000	460		

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SHEET NO. 5 OF 12

CALCULATED BY DPC DATE 21DEC76

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SCALE

STATION NO. D

DEPTH	PARAMETER	DATE 7DEC	8DEC	9DEC	10DEC	11DEC	AVERAGE VALUE	RANGE
0.5ft	TEMP, °C	7.5	6.5	6.0	5.5	6.0	6.3	5.5 - 7.5
	D.O., mg/l	13.4	12.4	13.2	13.3	12.2	12.9	12.2 - 13.4
	COND, μmho/cm	50	170	190	210	230	170	50 - 230
2.0ft	TEMP, °C	7.5	6.5		5.5			
	D.O., mg/l	13.0	12.3		13.1			
	COND, μmho/cm	80	175		210			
2.5ft	TEMP, °C					6.0		
	D.O., mg/l					12.2		
	COND, μmho/cm					230		
5.0ft	TEMP, °C			6.0				
	D.O., mg/l			13.2				
	COND, μmho/cm			210				

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SHEET NO. 6 OF 12

CALCULATED BY DPC DATE 21 DEC 76

CHECKED BY DATE

SCALE

STATION NO. D2

DEPTH	PARAMETER	DATE 7 DEC	8 DEC	9 DEC	10 DEC	11 DEC	AVERAGE VALUE	RANGE
0.5 ft	TEMP, °C	7.5	7.0	6.0	5.5	6.0	6.4	5.5 - 7.5
	D.O., mg/l	13.4	12.6	13.2	13.2	12.4	13.0	12.4 - 13.4
	COND, μmho/cm	155	180	225	190	220	194	155 - 225
2.0 ft	TEMP, °C	7.5	7.0				7.3	7.0 - 7.5
	D.O., mg/l	13.1	12.5				12.8	12.5 - 13.1
	COND, μmho/cm	165	180				173	165 - 180
3.0 ft	TEMP, °C	7.5	7.0				7.3	7.0 - 7.5
	D.O., mg/l	13.0	12.4				12.7	12.4 - 13.0
	COND, μmho/cm	170	175				173	170 - 175
4.0 ft	TEMP, °C	7.3	7.0	6.0	5.5	6.0	6.4	5.5 - 7.3
	D.O., mg/l	12.6	12.2	13.0	13.1	12.4	12.7	12.2 - 13.1
	COND, μmho/cm	205	180	230	230	220	213	180 - 230
5.0 ft	TEMP, °C	7.3	7.0				7.2	7.0 - 7.3
	D.O., mg/l	12.4	12.0				12.2	12.0 - 12.4
	COND, μmho/cm	290	190				240	190 - 290
5.5 ft	TEMP, °C	7.8	7.0	8 ft 6.0	8 ft 5.5	8 ft 6.0	DEPTH OF BOTTOM	
	D.O., mg/l	10.8	11.6	12.8	12.8	12.6		
	COND, μmho/cm	500	225	340	350	460		

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SHEET NO. 7 OF 12

CALCULATED BY DPC DATE 21 DEC 76

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SCALE

STATION NO. E1

DEPTH	PARAMETER	DATE 7 DEC	8 DEC	9 DEC	10 DEC	11 DEC	AVERAGE VALUE	RANGE
0.5 ft	TEMP, °C	7.5	7.0	6.5	6.0	6.5	6.7	6.0 - 7.5
	D.O., mg/l	13.6	12.4	13.0	12.7	11.8	12.7	11.8 - 13.6
	COND, μmho/cm	140	150	185	170	170	163	140 - 185
2.0 ft	TEMP, °C	7.5	7.0				7.3	7.0 - 7.5
	D.O., mg/l	13.2	12.3				12.8	12.3 - 13.2
	COND, μmho/cm	140	155				148	140 - 155
3.0 ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.7	12.1				12.4	12.1 - 12.7
	COND, μmho/cm	145	155				150	145 - 155
4.0 ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.6	12.0				12.3	12.0 - 12.6
	COND, μmho/cm	150	155				153	150 - 155
5.0 ft	TEMP, °C	7.3	7.5					
	D.O., mg/l	12.4	11.9					
	COND, μmho/cm	150	155					
6.0 ft	TEMP, °C		7.5	6.5	6.0	6.5	MID DEPTH	
	D.O., mg/l		11.8	12.8	12.4	11.8		
	COND, μmho/cm		160	185	180	175		
7.0 ft	TEMP, °C		7.5	6.0	6.0	6.5	DEPTH OF BOTTOM	
	D.O., mg/l		11.8	12.4	12.4	11.2		
	COND, μmho/cm		180	230	240	370		

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SHEET NO. 8 OF 12
CALCULATED BY DPC DATE 21DEC76
CHECKED BY DATE
SCALE

STATION NO. E_2

DEPTH	PARAMETER	DATE 7DEC	8DEC	9DEC	10DEC	11DEC	AVERAGE VALUE	RANGE
0.5ft	TEMP, °C	8.0	7.0	7.0	6.3	7.0	7.1	6.3 - 8.0
	D.O., mg/l	13.1	12.5	13.0	12.4	11.6	12.5	11.6 - 13.1
	COND, μmho/cm	65	115	150	160	160	130	65 - 160
2.0ft	TEMP, °C	7.8	7.0				7.4	7.0 - 7.8
	D.O., mg/l	13.0	12.2				12.6	12.2 - 13.0
	COND, μmho/cm	85	120				103	85 - 120
3.0ft	TEMP, °C	7.5	7.0			7.0	7.2	7.0 - 7.5
	D.O., mg/l	12.7	12.2			11.5	12.1	11.5 - 12.7
	COND, μmho/cm	90	120			160	123	90 - 160
4.0ft	TEMP, °C	7.5	7.0		4.5ft 6.3		6.9	6.3 - 7.5
	D.O., mg/l	12.4	12.1		12.3		12.3	12.1 - 12.4
	COND, μmho/cm	95	130		160		128	95 - 160
5.0ft	TEMP, °C	7.5	7.0	7.0				
	D.O., mg/l	12.2	12.0	12.6				
	COND, μmho/cm	100	135	160				
6.0ft	TEMP, °C		7.0					
	D.O., mg/l		12.0					
	COND, μmho/cm		145					
7.0ft	TEMP, °C		7.0			6.5		
	D.O., mg/l		11.9			11.4		
	COND, μmho/cm		150			160		

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JOB

SHEET NO. 9 OF 12

CALCULATED BY DPC DATE 21 DEC 76

CHECKED BY DATE

SCALE

STATION NO. E2

DEPTH	PARAMETER	DATE					AVERAGE VALUE	RANGE
		7 DEC	8 DEC	9 DEC	10 DEC	11 DEC		
8.0 ft	TEMP, °C		7.0					
	D.O., mg/l		11.9					
	COND, μmho/cm		155					
9.0 ft	TEMP, °C		7.0	6.5	6.3			
	D.O., mg/l		10.8	12.4	12.2			
	COND, μmho/cm		160	170	160			
10.0 ft.	TEMP, °C		7.5					
	D.O., mg/l		11.2					
	COND, μmho/cm		175					

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JOB

SHEET NO. 10 OF 12

CALCULATED BY DPC DATE 21 DEC 76

CHECKED BY DATE

SCALE

STATION NO. F

DEPTH	PARAMETER	DATE 7 DEC	8 DEC	9 DEC	10 DEC	11 DEC	AVERAGE VALUE	RANGE
0.5ft	TEMP, °C	7.8	7.5	7.0	6.5	6.5	7.1	6.5 - 7.8
	D.O., mg/l	13.0	12.4	12.7	11.9	11.3	12.3	11.3 - 13.0
	COND, μmho/cm	130	110	160	160	155	143	110 - 160
2.0ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.7	11.8				12.3	11.8 - 12.7
	COND, μmho/cm	130	120				125	120 - 130
3.0ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.4	11.6				12.0	11.6 - 12.4
	COND, μmho/cm	135	125				130	125 - 135
4.0ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.2	15.4				13.8	12.2 - 15.4
	COND, μmho/cm	140	130				135	130 - 140
5.0ft	TEMP, °C	7.5	7.5				7.5	—
	D.O., mg/l	12.1	15.4				13.8	12.1 - 15.4
	COND, μmho/cm	140	140				140	—
6.0ft	TEMP, °C	7.3					7.3	—
	D.O., mg/l	12.1					12.1	—
	COND, μmho/cm	140					140	—

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JOB

SHEET NO. 11 OF 12

CALCULATED BY DPC DATE 21 DEC 76

CHECKED BY DATE

SCALE

STATION NO. F

DEPTH	PARAMETER	DATE 7DEC	8DEC	9DEC	10DEC	11DEC	AVERAGE VALUE	RANGE
7.0ft	TEMP, °C	7.3					7.3	—
	D.O., mg/l	11.9					11.9	—
	COND, μmho/cm	140					140	—
8.0ft	TEMP, °C	7.5					7.5	—
	D.O., mg/l	11.9					11.9	—
	COND, μmho/cm	140					140	—
9.0ft	TEMP, °C	7.5					7.5	—
	D.O., mg/l	11.8					11.8	—
	COND, μmho/cm	140					140	—
10.0ft	TEMP, °C	7.5	7.5	6.5	6.5		7.0	6.5 - 7.5
	D.O., mg/l	11.8	15.4	12.0	11.7		12.7	11.7 - 15.4
	COND, μmho/cm	140	145	160	160		151	140 - 160
11.0ft	TEMP, °C	7.5					7.5	—
	D.O., mg/l	11.9					11.9	—
	COND, μmho/cm	140					140	—
12.0ft	TEMP, °C	7.5					7.5	—
	D.O., mg/l	11.8					11.8	—
	COND, μmho/cm	140					140	—

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JOB

SHEET NO 12 OF 12

CALCULATED BY DPC DATE 21 DEC 76

CHECKED BY DATE

SCALE

STATION NO. F

DEPTH	PARAMETER	DATE					AVERAGE VALUE	RANGE
		7 DEC	8 DEC	9 DEC	10 DEC	11 DEC		
13 0/t	TEMP, °C	7.5				6.5	7.0	6.5 - 7.5
	D.O., mg/l	11.9				11.2	11.6	11.2 - 11.9
	COND, μmho/cm	165				160	163	160 - 165
14.0 f+	TEMP, °C	7.5						
	D.O., mg/l	12.2						
	COND, μmho/cm	220						
15.0 f+	TEMP, °C		7.5					
	D.O., mg/l		15.4					
	COND, μmho/cm		150					
20 0 f+	TEMP, °C		7.5					
	D.O., mg/l		15.3					
	COND, μmho/cm		155					
22 f+	TEMP, °C				6.5			
	D.O., mg/l				11.5			
	COND, μmho/cm				160			
25 f+	TEMP, °C			6.5		6.5	DEPTH OF BOTTOM	
	D.O., mg/l			11.8		11.4		
	COND, μmho/cm			210		205		

TABLE A-2

IN SITU MEASUREMENTS OF DISSOLVED
OXYGEN, TEMPERATURE AND CONDUCTIVITY
VAAP, MARCH 1977, PHASE II

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ARMY MEDICAL CORPS V PHASE II

FIELD DATA

STATION NUMBER: 1

DEPTH	PARAMETER	DATE						AVG. VALUE	RANGE
		28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR		
	TEMPERATURE °C	9.1	8.0	9.5	10.0	13.0	11.0	10.1	8.0-13.0
SURFACE ½ ft	DISSOLVED OXYGEN mg/l	10.4	10.4	10.8	11.0	10.0	9.4	10.3	9.4-11.0
	CONDUCTIVITY µmho/cm	590	480	565	515	585	560	549	480-590
	TEMPERATURE °C	—	—	—	—	—	—	—	—
MID-DEPTH — ft	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—	—
	TEMPERATURE °C	8.5	8.5	10.5	10.0	12.5	11.0	10.2	8.5-12.5
BOTTOM ~ 3 ft	DISSOLVED OXYGEN mg/l	10.6	10.8	10.8	10.8	10.4	9.6	10.5	9.6-10.8
	CONDUCTIVITY µmho/cm	605	480	625	515	630	570	571	480-630
	DEPTH ft	4½	3	3	2	3	4	3	2-4½

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STATION NUMBER: 2

DEPTH	PARAMETER	DATE 28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR	AVG. VALUE	RANGE
	TEMPERATURE °C	11.3	8.5	9.5	10.0	12.0	12.0	10.6	8.5-12.0
SURFACE ½ ft	DISSOLVED OXYGEN mg/l	9.8	10.0	10.0	10.8	10.0	9.2	10.0	9.2-10.8
	CONDUCTIVITY µmho/cm	588	475	545	510	690	580	565	475-690
	TEMPERATURE °C	—	—	—	—	—	—	—	—
MID-DEPTH — ft	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—	—
	TEMPERATURE °C	10.2	8.5	10.0	10.8	11.5	11.5	10.4	8.5-11.5
BOTTOM ~3 ft	DISSOLVED OXYGEN mg/l	10.0	10.4	10.4	10.4	10.4	9.4	10.2	9.4-10.4
	CONDUCTIVITY µmho/cm	579	480	600	515	700	570	574	480-700
	DEPTH ft	4	3	3	3	2	4	3	2-4

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STATION NUMBER: 3

DEPTH	PARAMETER	DATE 28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR	AVG. VALUE	RANGE
	TEMPERATURE °C	12.0	10.0	9.5	10.3	11.5	12.5	11.0	9.5-12.5
SURFACE ½ ft	DISSOLVED OXYGEN mg/l	9.5	9.4	10.0	10.6	9.4	9.2	9.7	9.2-10.6
	CONDUCTIVITY µmho/cm	522	545	555	530	550	570	545	522-570
	TEMPERATURE °C	—	10.0	—	—	—	—	10.0	—
MID-DEPTH 3 ft	DISSOLVED OXYGEN mg/l	—	9.6	—	—	—	—	9.6	—
	CONDUCTIVITY µmho/cm	—	550	—	—	—	—	550	—
	TEMPERATURE °C	10.0	10.0	10.5	10.3	11.0	12.0	10.6	10.0-12.0
BOTTOM ~6 ft	DISSOLVED OXYGEN mg/l	9.8	9.6	10.8	10.4	9.8	9.2	9.9	9.2-10.8
	CONDUCTIVITY µmho/cm	588	540	595	530	570	560	564	530-595
	DEPTH ft	5	6	6	5	5	7	6	5-7

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ARMY MEDICAL CORPS V PHASE II

FIELD DATA

STATION NUMBER: 4

DEPTH	PARAMETER	DATE 28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR	AVG. VALUE	RANGE
	TEMPERATURE °C	12.5	10.0	10.0	10.0	11.5	12.0	11.0	10.0-12.5
SURFACE ½ft	DISSOLVED OXYGEN mg/l	9.4	9.2	10.0	10.6	9.4	9.2	9.6	9.2-10.6
	CONDUCTIVITY µmho/cm	550	535	560	530	525	580	547	525-580
	TEMPERATURE °C	—	10.0	—	—	—	—	10.0	—
MID-DEPTH 3 ft	DISSOLVED OXYGEN mg/l	—	9.6	—	—	—	—	9.6	—
	CONDUCTIVITY µmho/cm	—	540	—	—	—	—	540	—
	TEMPERATURE °C	11.0	10.0	10.5	10.3	11.0	12.0	10.8	10.0-12.0
BOTTOM ~5 ft	DISSOLVED OXYGEN mg/l	9.6	9.6	10.8	10.2	9.6	9.4	9.9	9.4-10.8
	CONDUCTIVITY µmho/cm	559	540	560	530	530	580	550	530-580
	DEPTH ft	6	6	3	5	6	6	5	3-6

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ARMY MEDICAL CORPS V PHASE II

FIELD DATA

STATION NUMBER: 5

DEPTH	PARAMETER	DATE 28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR	AVG. VALUE	RANGE
	TEMPERATURE °C	13.0	10.0	9.5	10.0	10.5	11.5	10.8	9.5-13.0
SURFACE ½ ft	DISSOLVED OXYGEN mg/l	10.6	9.0	10.4	10.4	9.8	9.0	9.9	9.0-10.6
	CONDUCTIVITY µmho/cm	400	540	590	550	530	540	525	400-590
	TEMPERATURE °C	—	—	—	—	—	—	—	—
MID-DEPTH — ft	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—	—
	TEMPERATURE °C	12.8	10.0	11.0	10.0	11.0	11.0	11.0	10.0-12.8
BOTTOM ~ 5 ft	DISSOLVED OXYGEN mg/l	10.6	9.2	10.5	9.8	9.8	9.0	9.8	9.0-10.6
	CONDUCTIVITY µmho/cm	410	540	610	550	525	525	527	410-610
	DEPTH ft	4	5	5	3	5	6	5	3-6

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STATION NUMBER: 6

DEPTH	PARAMETER	DATE 28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR	AVG. VALUE	RANGE
	TEMPERATURE °C	10.0	10.0	9.5	9.5	10.5	10.0	9.9	9.5-10.5
SURFACE ½ ft	DISSOLVED OXYGEN mg/l	11.2	10.0	11.4	10.8	10.0	10.0	10.6	10.0-11.4
	CONDUCTIVITY µmho/cm	230	400	350	415	360	300	343	230-415
	TEMPERATURE °C	—	—	—	—	—	—	—	—
MID-DEPTH — ft	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—	—
	TEMPERATURE °C	10.0	10.0	11.0	10.0	—	10.0	10.2	10.0-11.0
BOTTOM ~ 2 ft	DISSOLVED OXYGEN mg/l	10.8	10.4	11.6	10.6	—	10.4	10.8	10.4-11.6
	CONDUCTIVITY µmho/cm	330	420	425	465	—	330	394	330-465
	DEPTH ft	2	2	2	2	< 2	2	2	—

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STATION NUMBER: 7

DEPTH	PARAMETER	DATE 28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR	AVG. VALUE	RANGE
	TEMPERATURE °C	10.1	10.0	9.5	9.5	10.3	10.0	9.9	9.5-10.3
SURFACE ½ ft	DISSOLVED OXYGEN mg/l	10.8	11.0	11.0	11.4	10.0	10.4	10.8	10.0-11.4
	CONDUCTIVITY µmho/cm	246	260	330	330	345	310	304	246-345
	TEMPERATURE °C	—	9.5	—	9.5	10.0	—	9.7	9.5-10.0
MID-DEPTH 3 ft	DISSOLVED OXYGEN mg/l	—	11.0	—	11.2	10.8	—	11.0	10.8-11.2
	CONDUCTIVITY µmho/cm	—	290	330	340	340	—	325	290-340
	TEMPERATURE °C	9.2	9.0	10.0	9.5	10.0	10.0	9.6	9.0-10.0
BOTTOM ~6 ft	DISSOLVED OXYGEN mg/l	10.8	10.0	11.4	11.2	10.4	10.6	10.7	10.0-11.4
	CONDUCTIVITY µmho/cm	265	440	350	350	340	300	341	265-440
	DEPTH ft	6	7	5	6	6	7	6	5-7

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ARMY MEDICAL CORPS V PHASE II
FIELD DATA

STATION NUMBER: 8

DEPTH	PARAMETER	DATE 28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR	AVG. VALUE	RANGE
	TEMPERATURE °C	10.0	9.5	9.5	9.5	9.5	10.5	9.8	9.5-10.5
SURFACE ½ ft	DISSOLVED OXYGEN mg/l	11.2	11.2	11.2	10.4	10.4	10.4	10.8	10.4-11.2
	CONDUCTIVITY µmho/cm	250	235	290	280	270	310	273	235-310
	TEMPERATURE °C	—	9.5	9.5	9.5	9.5	10.0	9.6	9.5-10.0
MID-DEPTH ~5 ft	DISSOLVED OXYGEN mg/l	—	11.6	12.2	11.4	11.0	11.0	11.4	11.0-12.2
	CONDUCTIVITY µmho/cm	—	240	235	280	280	310	269	235-310
	TEMPERATURE °C	9.0	9.0	9.0	9.5	9.5	9.0	9.2	9.0-9.5
BOTTOM ~10 ft	DISSOLVED OXYGEN mg/l	11.1	10.8	11.6	11.2	10.8	10.0	10.9	10.0-11.6
	CONDUCTIVITY µmho/cm	240	260	290	265	270	210	256	210-290
	DEPTH ft	10	11	10	11	9	10	10	9-11

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ARMY MEDICAL CORPS V PHASE II

FIELD DATA

STATION NUMBER: 9

DEPTH	PARAMETER	DATE 28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR	AVG. VALUE	RANGE
SURFACE ½ ft	TEMPERATURE °C	9.5	9.0	8.5	9.5	9.5	10.0	9.3	8.5-10.0
	DISSOLVED OXYGEN mg/l	11.8	11.4	12.0	10.0	10.4	10.4	11.0	10.0-12.0
	CONDUCTIVITY µmho/cm	179	195	215	205	210	285	215	179-285
MID-DEPTH ~6 ft	TEMPERATURE °C	—	8.5	8.5	9.0	9.5	9.0	8.9	8.5-9.5
	DISSOLVED OXYGEN mg/l	—	12.0	12.0	11.4	11.0	11.0	11.5	11.0-12.0
	CONDUCTIVITY µmho/cm	—	190	230	205	210	210	209	190-230
BOTTOM ~12 ft	TEMPERATURE °C	8.5	8.0	8.5	8.5	9.0	8.5	8.5	8.0-9.0
	DISSOLVED OXYGEN mg/l	12.0	11.6	11.6	11.0	10.6	10.4	11.2	10.4-12.0
	CONDUCTIVITY µmho/cm	180	190	240	200	210	210	205	180-240
	DEPTH ft	12	12	13	11	11	15	12	11-15

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ARMY MEDICAL CORPS V PHASE II

FIELD DATA

STATION NUMBER: 10

DEPTH	PARAMETER	DATE 28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR	AVG. VALUE	RANGE
	TEMPERATURE °C	8.5	—	—	—	—	—	8.5	—
SURFACE ½ ft	DISSOLVED OXYGEN mg/l	11.0	—	—	—	—	—	11.0	—
	CONDUCTIVITY µmho/cm	190	—	—	—	—	—	190	—
	TEMPERATURE °C	—	—	—	—	—	—	—	—
MID-DEPTH — ft	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—	—
	TEMPERATURE °C	7.5	—	—	—	—	—	7.5	—
BOTTOM 12 ft	DISSOLVED OXYGEN mg/l	11.0	—	—	—	—	—	11.0	—
	CONDUCTIVITY µmho/cm	200	—	—	—	—	—	200	—
	DEPTH ft	12	—	—	—	—	—	12	—

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ARMY MEDICAL CORPS V PHASE II

FIELD DATA

STATION NUMBER: 11

DEPTH	PARAMETER	DATE 28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR	AVG. VALUE	RANGE
SURFACE ½ ft	TEMPERATURE °C	10.5	10.0	10.0	9.0	11.0	—	10.1	9.0-11.0
	DISSOLVED OXYGEN mg/l	11.0	11.2	11.6	11.6	10.0	—	11.1	10.0-11.6
	CONDUCTIVITY µmho/cm	180	200	230	220	240	—	214	180-240
MID-DEPTH — ft	TEMPERATURE °C	—	—	—	—	—	—	—	—
	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—	—
BOTTOM ~7 ft	TEMPERATURE °C	8.5	—	9.5	9.0	9.0	—	9.0	8.5-9.5
	DISSOLVED OXYGEN mg/l	10.8	—	11.8	11.6	10.6	—	11.2	10.6-11.8
	CONDUCTIVITY µmho/cm	199	—	235	225	230	—	222	199-235
	DEPTH ft	8	—	8	8	5	—	7	5-8

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ARMY MEDICAL CORPS V PHASE II

FIELD DATA

STATION NUMBER: 12

DEPTH	PARAMETER	DATE 28 FEB	1 MAR	2 MAR	3 MAR	4 MAR	5 MAR	AVG. VALUE	RANGE
	TEMPERATURE °C	12.0	—	—	—	—	—	12.0	—
SURFACE ½ ft	DISSOLVED OXYGEN mg/l	12.0	—	—	—	—	—	12.0	—
	CONDUCTIVITY µmho/cm	215	—	—	—	—	—	215	—
	TEMPERATURE °C	—	—	—	—	—	—	—	—
MID-DEPTH — ft	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—	—
	TEMPERATURE °C	—	—	—	—	—	—	—	—
BOTTOM < 1 ft	DISSOLVED OXYGEN mg/l	—	—	—	—	—	—	—	—
	CONDUCTIVITY µmho/cm	—	—	—	—	—	—	—	—
	DEPTH ft	<1	—	—	—	—	—	<1	—

AD-A055 901 WATER AND AIR RESEARCH INC GAINESVILLE FLA F/G 6/6
WINTER FIELD SURVEYS AT VOLUNTEER ARMY AMMUNITION PLANT, CHATTA--ETC(U)
APR 78 J H SULLIVAN, H D PUTNAM, M A KEIRN DAMD17-75-C-5049
UNCLASSIFIED NL

2 OF 2
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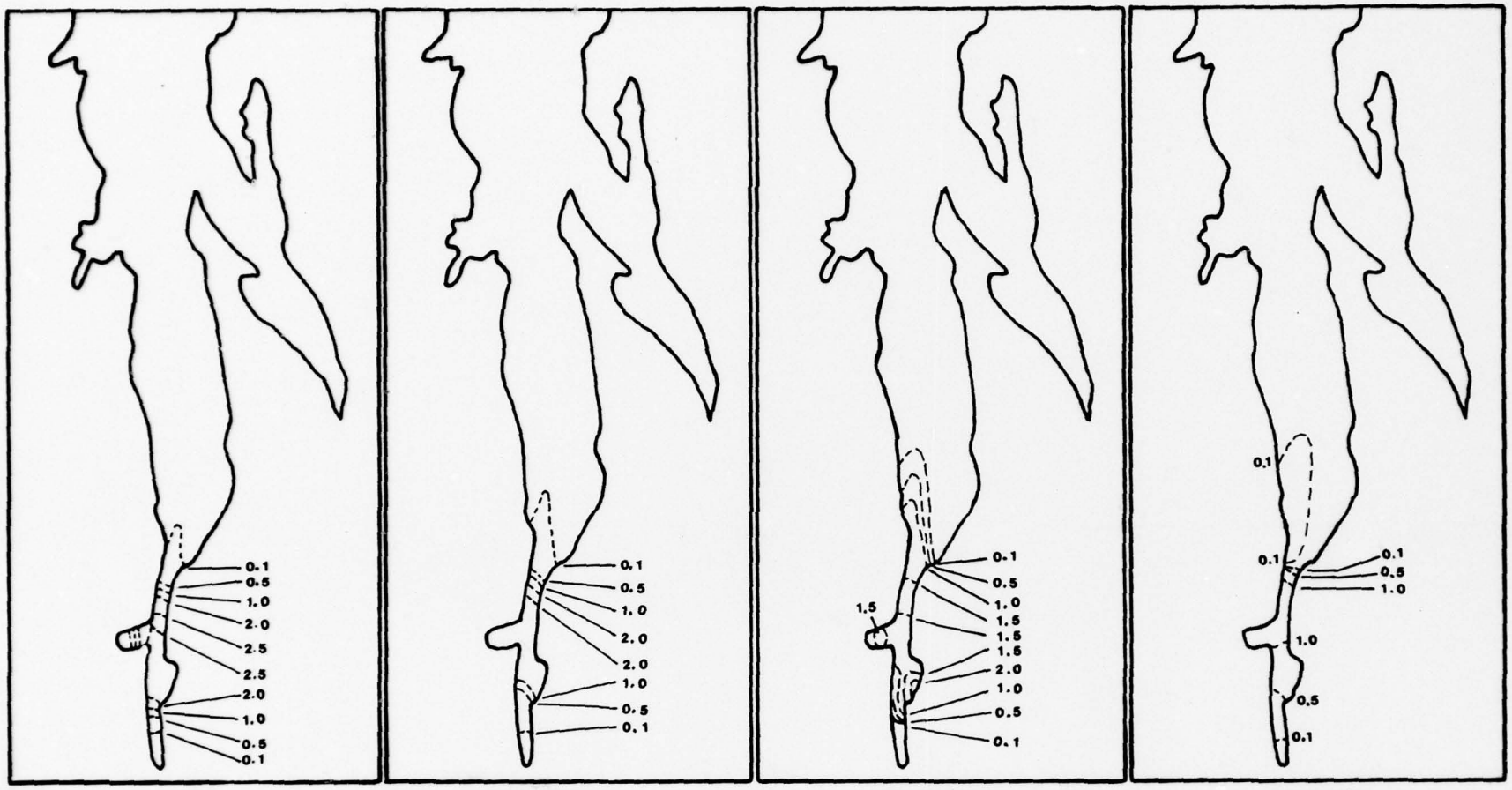


12/8/76
0916-1025

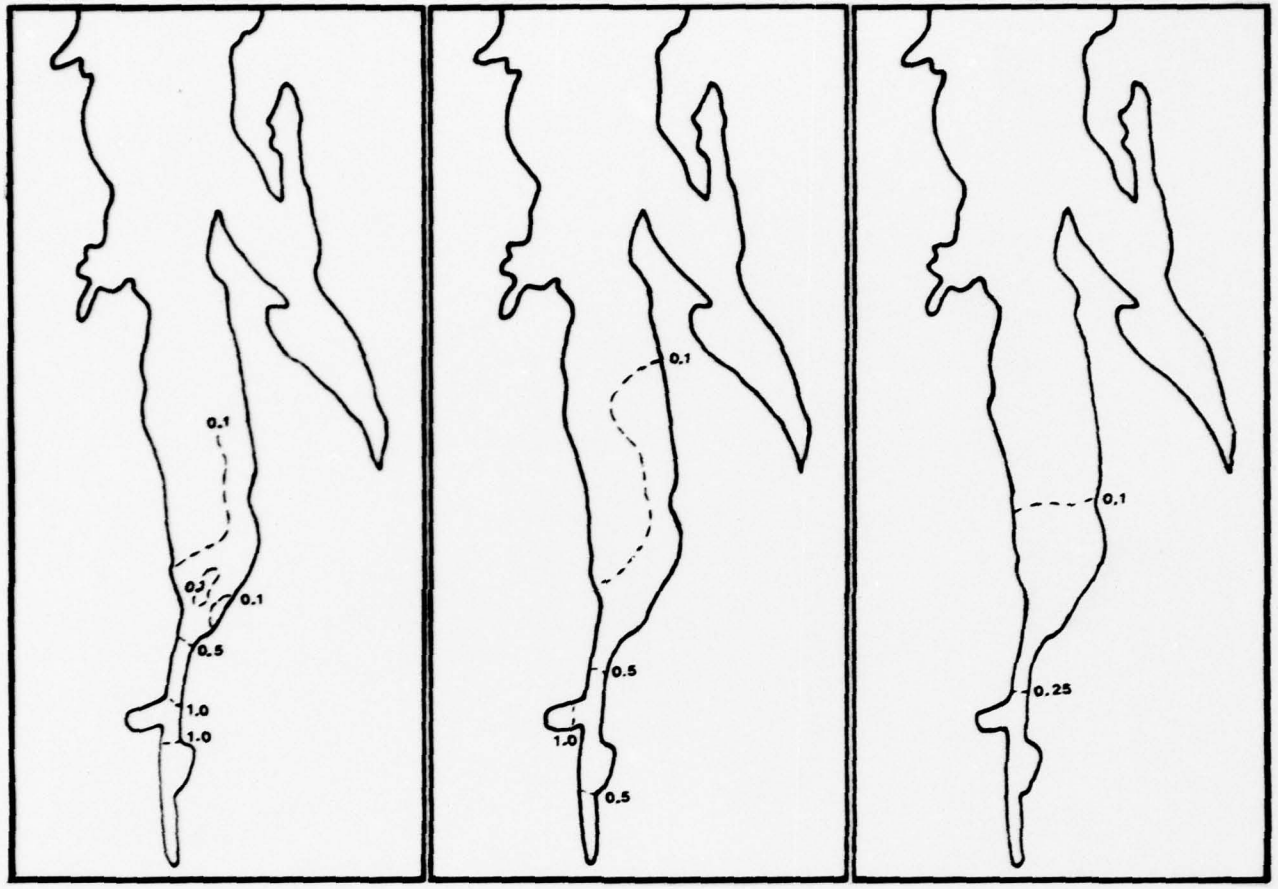
12/8/76
1400-1512

12/9/76
0925-1100

12/9/76
1600-1721

FIGURE A-1

RHODAMINE B DYE CONCENTRATION - SURFACE
(ppb by weight)

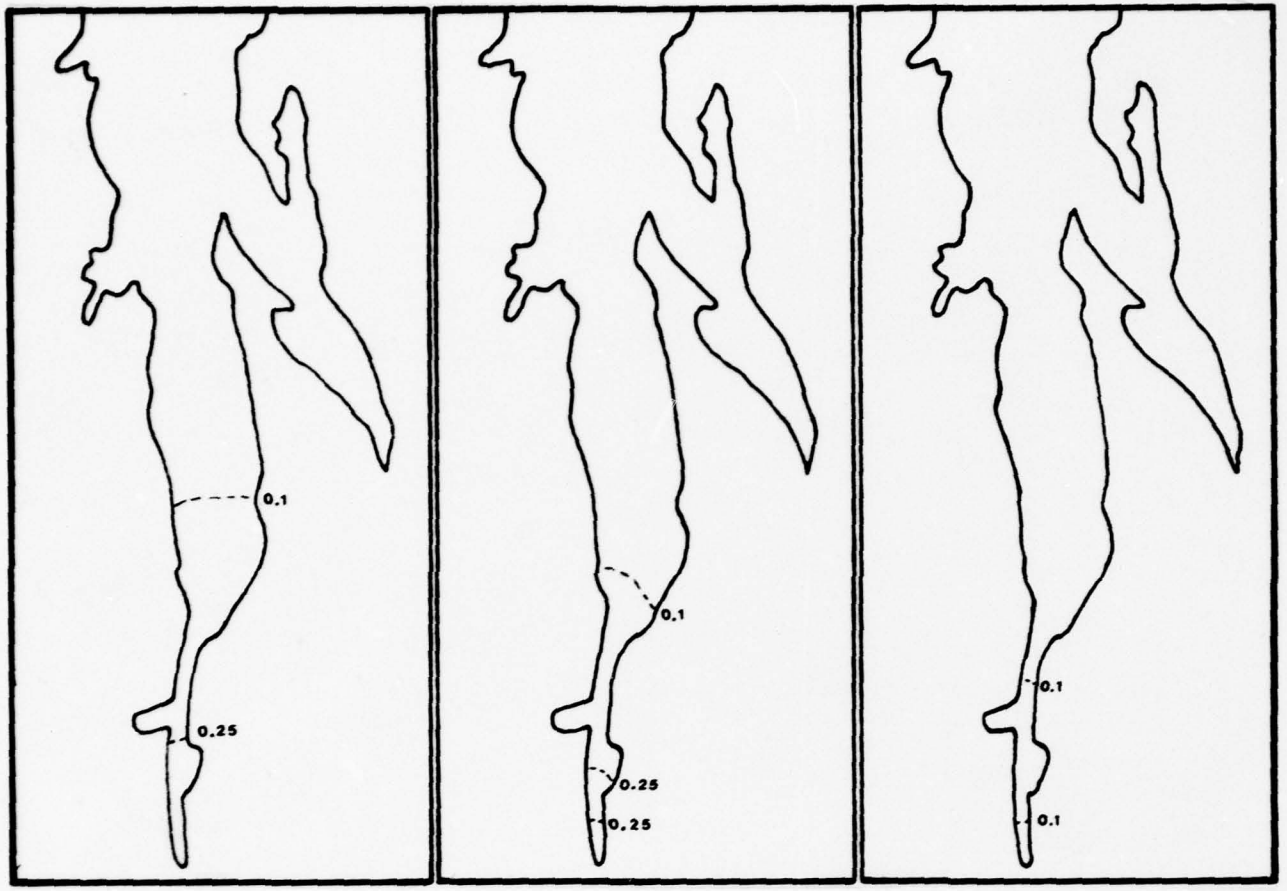


12/10/76
0916-1148

12/10/76
1530-1636

12/11/76
0838-1009

FIGURE A-1. RHODAMINE B DYE CONCENTRATION-SURFACE
(ppb by weight)



12/11/76
1216-1320

12/12/76

12/13/76

FIGURE A-1 RHODAMINE B DYE CONCENTRATIONS-SURFACE
(ppb by weight)

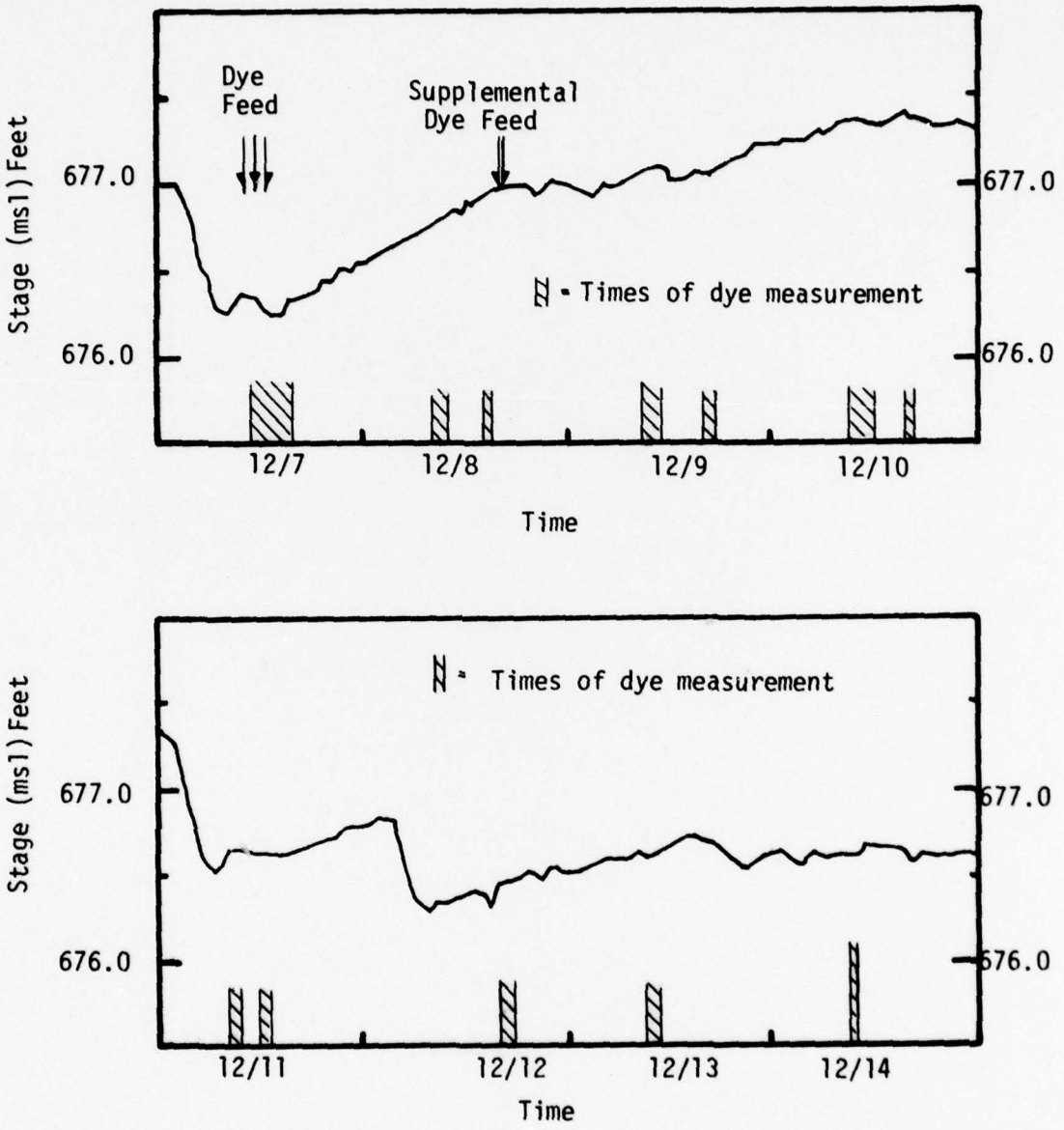
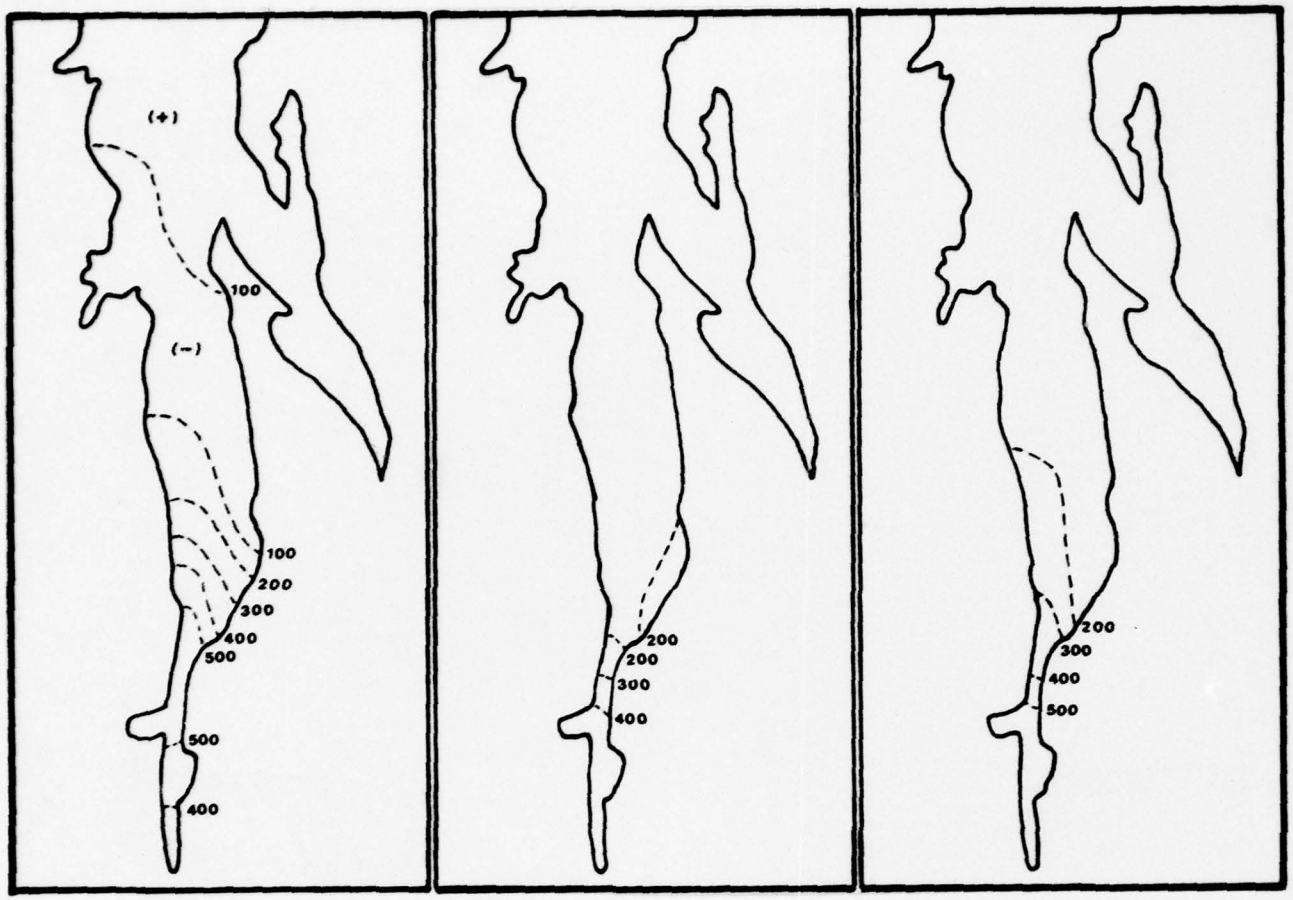
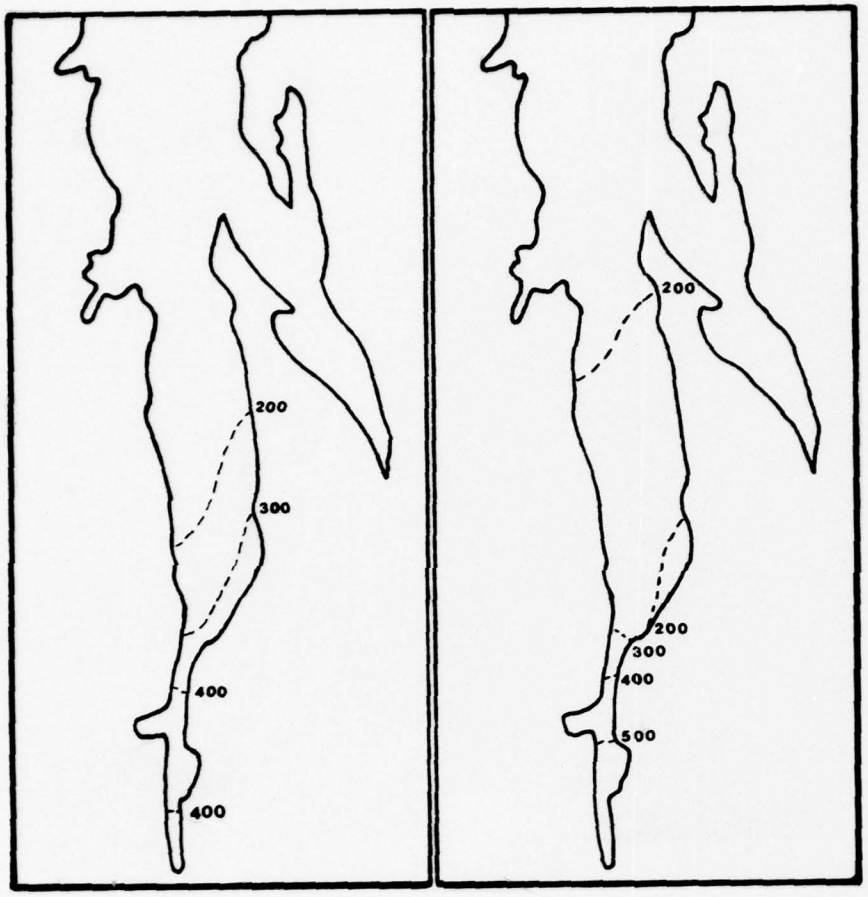


FIGURE A-2. CHICKAMAUGA RESERVOIR STAGE VS. TIME.



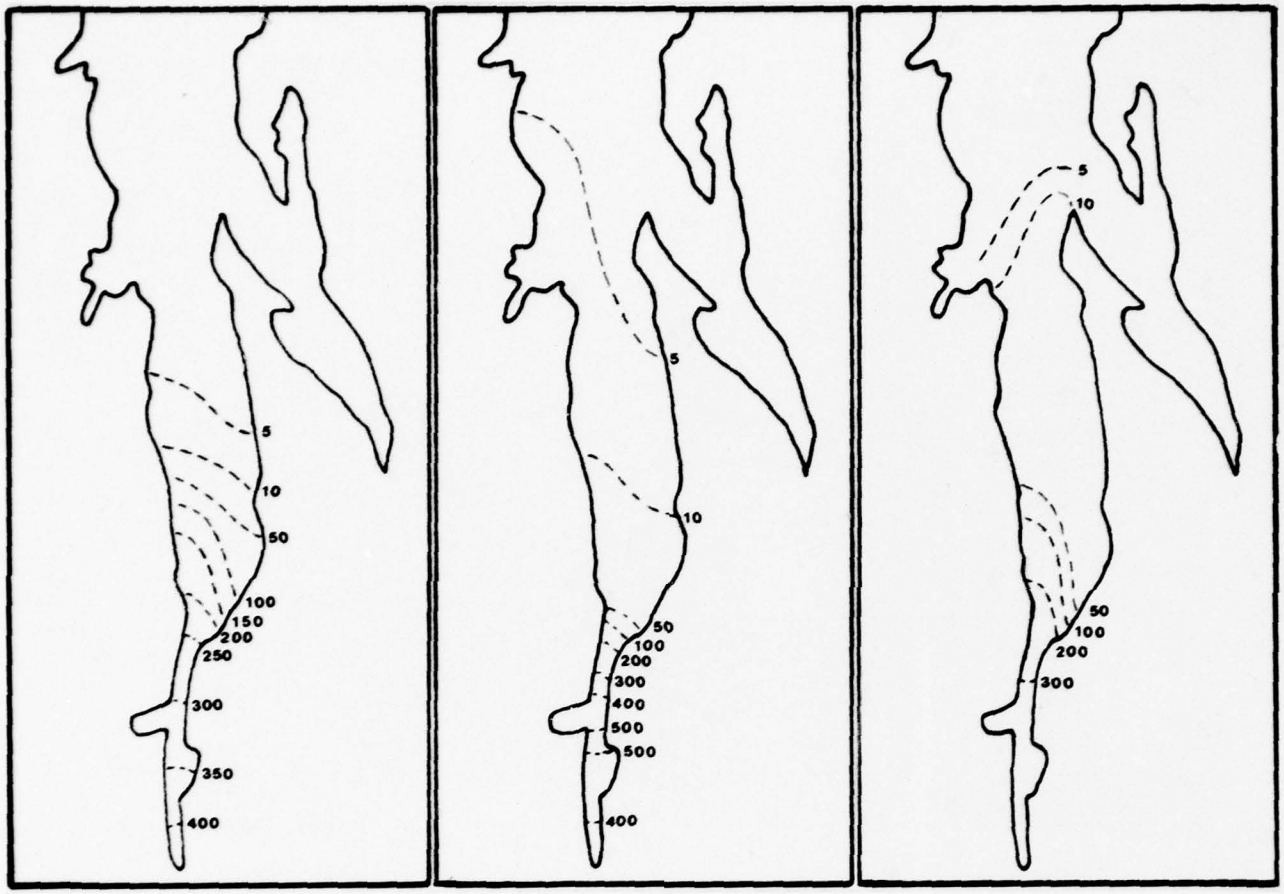
12/7/76 12/8/76 12/9/76

FIGURE A-3. CONDUCTIVITY-SURFACE (μmhos/cm)



12/10/76 12/11/76

FIGURE A-3. CONDUCTIVITY-SURFACE (μmhos/cm)



12/7/76 12/8/76 12/9/76

FIGURE A-4. TOTAL MUNITIONS CONCENTRATION, µg/l.

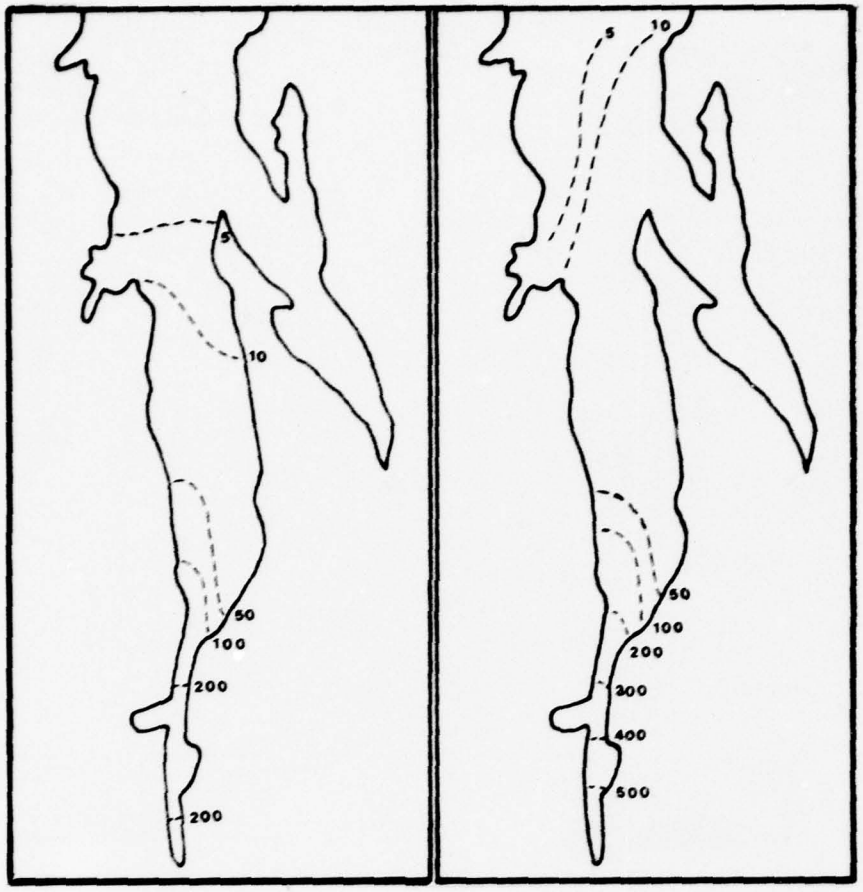


FIGURE A-4. TOTAL MUNITIONS CONCENTRATION, µg/l.

APPENDIX B

ANALYTICAL PROCEDURES

LIST OF TABLES
APPENDIX B - ANALYTICAL PROCEDURES

ANALYTICAL PROCEDURES

Standard Methods or EPA-approved procedures were employed to characterize the background water quality (see Table B-1 for specific procedures). Ammonia nitrogen and total Kjeldahl nitrogen were analyzed on a Technicon Autoanalyzer II using the manufacturer's specified methodology. The block digestor and the salicylate-nitroprusside colorimetric methods are currently being researched by the EPA and are approved for use in NPDES permit monitoring (Gales, EPA, 1977 personal communication). Analytical procedures (Table B-2) utilized on sediment samples were primarily from Chemistry Laboratory Manual Bottom Sediments (EPA, 1969).

Munitions Analysis

The water samples were collected in amber glass reagent bottles that were pre-rinsed in acetone. The samples were refrigerated until analysis, which consisted of extraction, concentration and gas-liquid chromatography. Sediment samples were collected in glass jars that had been pre-rinsed with acetone. Saran wrap was used as a cap liner.

Extraction of Water Samples, December 1976. A sample of 250 ml was measured into a clean 500 ml separatory funnel equipped with a Teflon stop-cock. Seventy-five ml of ethyl acetate (pesticide grade) was added, the flask stoppered, and shaken for 2 to 3 minutes. The layers were allowed to separate and the lower (water) layer drained into a second 500 ml separatory funnel and again extracted with 50 ml ethyl acetate. The water layer was discarded. The extracts were combined and filtered through a plug of cotton previously wetted with ethly acetate. The separatory funnels were rinsed with an additional 10 ml of ethyl acetate and filtered through the cotton plug. The ethyl acetate was evaporated to a volume of 2.5 ml under reduced pressure with the flask temperature not exceeding 40°C.

Extraction of Water Samples, March 1977. Same procedure except 500 ml samples were used.

Extraction of Sediment Samples. In order to dry the wet sediments, 80 gm of sodium sulfate was added to 20 gm of wet sediment. This was then packed into a chromatographic column and extracted for one hour with ethyl acetate. The extraction was followed by evaporation of the ethyl acetate extract to a volume of 5.0 ml under the same conditions as described earlier. Clean-up techniques are discussed in the recovery studies section of this procedure.

Chromatography of Extracts, December 1976. Samples were chromatographed on a 5 1/2 ft. x 1/8 in. silanized glass column packed with 2% OV 101/3% QF1 on 100/120 mesh Gas Chrom Q. A Varian Model 1840 Gas Chromatograph with electron capture (EC) and Thermionic (Alkali Flame Ionization Detector) (AFID) detectors was chosen. The readout was obtained by using a Varian Model 285 Electronic Integrator which was recorded permanently by a Beckman 1 mv, 10 inch scale recorder. Peak areas were automatically printed by integrator. Electron capture was chosen as the prime detector with AFID as back-up and confirmation detector.

TABLE B-1

SUMMARY OF ROUTINE LABORATORY ANALYTICAL PROCEDURES
FOR WATER SAMPLES

Parameter	Procedure
Chloride	Standard Methods, 408B: Mercuric Nitrate Method, p. 304.
Total Hardness	Standard Methods, 309B: EDTA Titrimetric Method, p. 202.
Sulfate	Standard Methods, 427C: Turbidimetric Method, $BaCl_2$, p. 496.
Ammonia Nitrogen	Technicon Corp. Industrial Method #239-74 W/A: Automated colorimetric salicylate-nitroprusside method, 1976.
Total Kjeldahl Nitrogen	EPA, STORET #00625: Acid Digestion, Distillation, Nesslerization, p. 175. or Technicon Corp. Industrial Method #376-75-W/A: Acid digestion on BD-20, 1975. Technicon Corp. Industrial Method #329-74-W/A: Automated colorimetric salicylate-nitroprusside method, 1976.
Nitrate-Nitrite Nitrogen	EPA, STORET #00630: Automated Cadmium Reduction Method, p. 207.

EPA 1974, Manual of Methods for Chemical Analysis of Water and Wastes.

Standard Methods for the Examination of Water and Wastewater, 14th Ed., 1975, APHA, AWWA, WPCF.

Technicon Industrial Systems; Tarrytown, New York.

TABLE B-2

SUMMARY OF ROUTINE LABORATORY ANALYTICAL PROCEDURES
FOR SEDIMENT SAMPLES

Parameter	Procedure
Total Kjeldahl Nitrogen	Bottom Sediments - Great Lakes; Acid Digestion, Distillation, and Titration with 0.02N H_2SO_4, p. 38.
Nitrate-Nitrite Nitrogen	Bottom Sediments - Great Lakes: Acid Digestion, p. 32. EPA, STORET #00630: Automated Cadmium Reduction Method, p. 207.
Total Solids	Bottom Sediments - Great Lakes: Gravimetric Method, p. 85.
Total Volatile Solids	Bottom Sediments - Great Lakes: Gravimetric Method, p. 85.

Chemistry Laboratory Manual Bottom Sediments, EPA 1969, compiled by Great Lakes Region Committee on Analytical Methods.

EPA 1974, Manual of Methods for Chemical Analysis of Water and Wastes.

An alternate column used for confirmatory information was a 4 ft. x 1/7 in. glass column packed with 8 percent UCW 98 on 80/100 mesh Gas Chrom Q. Instrument conditions for both columns and detectors were:

Column temperature:	175°C. isothermally
Injector temperature:	220°C
Detector temperature:	220°C
Carrier gas:	Nitrogen @ 70 ml/min.
Electrometer setting:	10^{-10} afs at 1 x attenuation into integrator with appropriate attenuation setting for recorder.

Five µl injections of sample extracts and standards were first injected onto the 2% OV 101/3% AF1 column using the EC Detector. Peaks corresponding to standards were noted and the areas compared. Samples and standards were next injected onto USW-98 column and like comparisons were made. Likewise, samples and standards were injected onto the 2% OV 101/3% QF1 column using the Thermionic or Alkali Flame Ionization Detector. Again, peaks corresponding to the standards were noted and the areas were compared. Sample peaks which did not elute at the same times as the standards on both sets of columns and detectors were rejected, and only those that were peaks confirmed on both sets were quantitated. Quantification was with the 2% OV 101/3% QF1 column with the EC detector.

Chromatography of Extracts, March 1977. Same procedure and conditions as above with the following exceptions:

Electron capture detector with a standing current of 100% at 16 x 10^{-10}
Attenuation set at 8 x 10-10
Injection volume of 4.0 µl
Column temperature, 165°C
Injector temperature, 200°C

Recovery Studies, December 1976. Recovery studies for water samples were made by addition of standards to tap water and carrying through entire extraction, concentration and GC procedures. Standards were prepared in acetone solution prior to addition to water. Previous recovery studies had been made by addition of standards prepared in ethyl acetate solution, however, it was thought that this might bias the study since ethyl acetate would be used for subsequent extraction. Hence, the selection of water soluble acetone as solvent would negate any positive effect which the standard solvent may have had.

Since previous studies had shown levels of less than 1 ppm TNT and analogs in water samples, spiking was made at and below this level as follows:

500 ml water was used in all cases.

COMPONENT	QUANTITY ADDED	QUANTITY RECOVERED	% RECOVERY
2, 4 DNT	500 µg	480	96
	250 µg	230	92
	100 µg	90	90
2, 6 DNT	200 µg	470	94
	250 µg	240	96
	100 µg	92	92
1, 3 DNB	500 µg	480	96
	250 µg	225	90
	100 µg	94	94
2, 4, 6 TNT	500 µg	460	92
	250 µg	230	92
	100 µg	95	95

SPIKING STUDIES, WATER SAMPLES

250 ml sample used

SAMPLE NO.	COMPONENT	µg PRESENT	µg ADDED	TOTAL RECOVERY	% RECOVERY
AB 21	2,4 DNT	0	25	24	96
	2,6 DNT	0	25	21	84
	TNT	0	25	25	100
	1,3 DNB	0	25	22	88
AB 110	2,4 DNT	55	25	70	88
	2,6 DNT	24	25	45	92
	TNT	69	25	95	101
	1,3 DNB	1	25	22	85
AB 114	2,4 DNT	6	25	30	97
	2,6 DNT	3	25	25	89
	TNT	3	25	24	86
	1,3 DNB	0	25	23	92
AB 37	2,4 DNT	0	62	59	95
	2,6 DNT	0	62	63	102
	TNT	0	62	60	97
	1,3 DNB	0	62	58	94
AB 103	2,4 DNT	51	62	105	93
	2,6 DNT	26	62	81	92
	TNT	19	62	75	93
	1,3 DNB	1.5	62	60	94
AB 204	2,4 DNT	28	62	82	91
	2,5 DNT	15	62	73	95
	TNT	15	62	75	97
	1,3 DNB	1.5	62	58	91

Sediment samples were column extracted with ethyl acetate after adding sufficient anhydrous sodium sulfate to absorb moisture and yield apparently dry product. Samples thus extracted contained too many interfering substances to be run directly on GLC and, therefore, were subjected to florisil column cleanup.

Elution of florisil with ethyl acetate not only removed the TNT compounds, but most of the interfering substances as well. It was found that elution of florisil column with 6% ethyl ether in hexane gave quantitative recovery of the compounds of interest while leaving most of the extraneous materials on the column. This procedure was adopted for cleanup.

Overall recovery averaging approximatelv 80% was not as good as with water samples. A more efficient extraction procedure would improve recovery.

Recovery of Standards added to Anhydrous Sodium Sulfate

COMPOUND	QUANTITY ADDED	QUANTITY RECOVERED	% RECOVERY
2,4 DNT	100 µg	94	94
2,6 DNT	100 µg	90	90
TNT	100 µg	95	95
2,4 DNT	250 µg	230	92
2,6 DNT	250 µg	225	90
TNT	250 µg	235	94

Indicated recovery ≈ 92%.

Recovery of Standards from Florisil Cleanup Procedure

COMPOUND	QUANTITY ADDED	QUANTITY RECOVERED	% RECOVERY
2,4 DNT	100 µg	96	96
2,6 DNT	100 µg	95	95
TNT	100 µg	98	98
2,4 DNT	250 µg	240	96
2,6 DNT	250 µg	230	92
TNT	250 µg	240	96

Recovery from Spiked Samples of Sediment

SAMPLE NO.	COMPONENT	µg PRESENT	µg ADDED	TOTAL RECOVERY	% RECOVERY
W4-1	2,4 DNT	0	25	21	84
	2,6 DNT	0	25	20	80
	TNT	0	25	22	88
W4-10	2,4 DNT	3	25	22	79
	2,6 DNT	2	25	21	78
	TNT	5	25	26	87
W4-4	2,4 DNT	0	50	40	80
	2,6 DNT	0	50	38	76
	TNT	0	50	42	84
W4-6	2,4 DNT	0	50	43	86
	2.6 DNT	0	50	42	84
	TNT	3	50	42	79

Recovery of standards added to samples and then put through extraction and cleanup procedures was not as good as extraction and cleanup without the presence of sample, thus indicating that the TNT compounds present some difficulty in extraction from sediment samples. Overall recovery indicated recovery is approximately 80%.

Recovery Studies, March 1977. Standards were prepared in acetone solution by diluting stock standard (1 mg/ml) with acetone to 100 µg/ml or 0.1 µg per µl. The combination standard then contained 0.1µg/µl of each of the following:

1, 3 DNB
1, 3, 5 TNB
2, 4 DNT
2, 6 DNT
2, 4, 6 TNT

Five hundred ml of distilled water was added to each of three separatory funnels and combination standard was added.

100 1 (10 µg) to No. 1
500 1 (50 µg) to No. 2
2.5 ml (250 µg) to No. 3

Contents of each funnel was mixed, the funnel stoppered and allowed to stand 1 hour. Contents of each funnel was then extracted and taken through same cleanup concentration and chromatography as were samples.

Recoveries were:

Component	Quantity Added µg	Quantity Recovered µg	Percent Recovered
1,3 DNB	10	9.1	91
	50	46.8	94
	250	240	96
1,3,5 TNB	10	9.0	90
	50	47.0	94
	250	235.0	94
2,4 DNT	10	9.5	95
	50	43.0	86
	250	230.0	92
2,6 DNT	10	9.3	93
	50	48.0	96
	250	232.0	93
2,4,6 TNT	10	9.2	92
	50	51.1	102
	250	242.0	97

Studies were also made with spiked samples similar to above except that known increments of standards were added to actual samples. Two hundred ml samples were spiked with varying amounts of standards using same procedures as with distilled water. These were extracted, concentrated, run through cleanup and chromatographed same as original samples with the following results:

Component	1,3 DNB	1,3,5 TNB	2,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally found (µg)	3.1	12.5	-	1.7	14.1
Amount added (µg)	10.0	10.0	10.0	10.0	10.0
Total present (µg)	13.1	12.5	10.0	11.7	24.1
Assay (µg)	12.3	12.3	9.1	10.5	22.2
% Recovery	93.9	98.4	91.0	89.7	92.1

Component	1,3 DNB	1,3,5 TNB	2,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally found (µg)	-	8.8	1.1	2.2	22.6
Amount added (µg)	10.0	10.0	10.0	10.0	10.0
Total present (µg)	10.0	18.8	11.1	12.2	32.6
Assay (µg)	9.3	18.0	10.3	11.1	31.2
% Recovery	93.0	95.7	92.8	91.0	95.7

Component	1,3 DNB	1,3,5 TNB	2,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally found (µg)	-	0.2	-	6.5	1.0
Amount added (µg)	5.0	5.0	5.0	5.0	5.0
Total present (µg)	5.0	5.2	5.0	11.5	6.0
Assay (µg)	5.1	4.8	4.7	10.7	5.8
% Recovery	102.0	92.3	94.0	93.0	96.7

Component	1,3 DNB	1,3,5 TNB	3,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally found (µg)	-	-	1.5	0.7	1.5
Amount added (µg)	5.0	5.0	5.0	5.0	5.0
Total present (µg)	5.0	5.0	6.5	5.7	6.5
Assay (µg)	4.9	4.8	6.2	5.6	6.6
% Recovery	98.0	96.0	95.4	98.2	101.5

Component	1,3 DNB	1,3,5 TNB	3,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally found (µg)	-	-	20.8	15.0	5.9
Amount added (µg)	10.0	10.0	10.0	10.0	10.0
Total present (µg)	10.0	10.0	30.8	25.0	15.9
Assay (µg)	9.7	9.5	27.9	23.2	15.1
% Recovery	97.0	95.0	90.6	92.8	95.0

Sediment samples were column extracted with ethyl acetate after adding sufficient anhydrous sodium sulfate to absorb moisture and yield an apparently dry product. Samples thus extracted contained too many interfering substances to be run directly on GLC and, therefore, were subjected to florisil cleanup, eluting with 6% ether in hexane. The areas of the plate containing the compounds of interest were then removed, extracted with ethyl acetate. This cleaned up sample was then adjusted in volume and quantitated using GLC.

Results of spiking studies are given below:

Component	1,3 DNB	1,3,5 TNB	2,4 DNT	2,6 DNT	2,4,6 TNT
Amount originally present (µg)	1.1	6.4	-	0.8	4.7
Amount added (µg)	2.5	2.5	2.5	2.5	2.5
Total present (µg)	3.6	8.9	2.5	3.3	7.2
Assay (µg)	3.0	7.0	2.0	2.5	6.0
% Recovery	83.3	78.7	80.0	75.8	83.3

Component	1,3,5 TNB	2,4,6 TNT
Amount originally present (µg)	15.5	8.6
Amount added (µg)	5.0	5.0
Total present (µg)	20.5	13.6
Assay (µg)	16.5	11.0
% Recovery	80.5	80.9

Component	1,3,5 TNB	2,4,6 TNT
Amount originally present (µg)	6.5	6.2
Amount added (µg)	5.0	5.0
Total present (µg)	11.5	11.2
Assay (µg)	9.0	9.5
% Recovery	78.3	84.8

Because of several factors such as extraction efficiency, additional cleanup steps and handling losses, overall recovery from sediment samples averages about 80% whereas recovery from water samples was considerably better.

APPENDIX C

WATER QUALITY AND VAAP EFFLUENT ANALYSES

LIST OF TABLES
APPENDIX C

WATER MUNITIONS TABLE C-1

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MUNITIONS LEVELS VAAP EFFLUENT
MARCH 1977

Water and Air Research, Inc.

PROJECT ANALYTICAL DATA SUMMARY

ARMY MEDICAL CORPS V
Project

PHASE II
Project Phase

VOLUNTEER ARMY AMMUNITION PLANT

SAMPLED MARCH 5-25, 1977

SAMPLING DATE	Value in µg/l							
	1,3-DNB	1,3,5-TNB	2,4-DNT	2,6-DNT	2,4,6-TNT			TOTAL
3/5-7/77	<0.25	<0.75	580	269	106			956
3/8/77	<0.25	<0.75	529	53.3	92.8			676
3/9/77	<0.25	8.2	<0.10	42.9	151			202
3/10/77	<0.25	<0.75	1040	223	198			1460
3/11/77	<0.25	<0.75	1520	416	426			2370
3/12-14/77	<0.25	<0.75	866	188	323			1380
3/15/77	<0.25	<0.75	758	153	387			1300
3/16/77	<0.25	<0.75	261	28.8	234			525
3/17/77	<0.25	<0.75	90.7	10.6	55.4			158
3/18/77	<0.25	9.8	111	46.6	45.7			213
3/19-21/77	<0.25	<0.75	167	69.3	83.4			321
3/22/77	<0.25	<0.75	113	38.5	98.1			251
3/23/77	<0.25	<0.75	9.4	7.8	69.8			88.0
3/24/77	<0.25	<0.75	161	55.4	70.9			288
3/25/77	<0.25	<0.75	<0.10	86.8	62.3			150

Comments Composite effluent samples taken by VAAP; 24 hour composites excepting the weekend composites.

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WATER MUNITIONS

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TABLE C-1 (CONT.)

Water and Air Research, Inc.

PROJECT ANALYTICAL DATA SUMMARY

ARMY MEDICAL CORPS V
Project

PHASE II
Project Phase

VOLUNTEER ARMY AMMUNITION PLANT

SAMPLED MARCH 28, 1977 TO APRIL 1, 1977

SAMPLING DATE	Value in ug/l							
	1,3-DNB	1,3,5-TNB	2,4-DNT	2,6-DNT	2,4,6-TNT			TOTAL
3/28/77	<0.25	<0.75	121	85.8	90.4			298
3/29/77	<0.25	<0.75	<0.10	53.4	121			176
3/30/77	<0.25	<0.75	<0.10	0.6	1.1			≤2.80
3/31/77	<0.25	<0.75	<0.10	47.8	152			201
4/1/77	<0.25	<0.75	137	87.2	245			470

Comments Composite effluent samples taken by VAAP; 24 hour composites excepting the weekend composites.

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TABLE C-2

VAAP EFFLUENT FLOW, MARCH 1977

Date	Flow, mgd
1	7.0
2	6.5
3	3.2
4	6.2
5	7.8
6	7.8
7	7.8
8	5.3
9	4.2
10	3.7

Date	Flow, mgd
11	3.5
12	7.7
13	7.7
14	7.7
15	7.9
16	4.9
17	4.9
18	5.2
19	5.0
20	5.0

Date	Flow, mgd
21	5.0
22	6.6
23	7.3
24	6.0
25	6.0
26	5.6
27	5.5
28	5.5
29	4.6
30	---
31	5.6

TABLE C-3

VAAP MUNITIONS CHEMISTRY
RANGE OF VALUES

Water and Air Research, Inc.

PROJECT ANALYTICAL DATA SUMMARY

ARMY MEDICAL CORPS V — **Project**

PHASE II — **Project Phase**

VOLUNTEER ARMY AMMUNITION PLANT

SAMPLED March 1-5, 1977

STATION NUMBER	RANGE of Values in µg/l							
	1,3-DNB	1,3,5-TNB	2,4-DNT	2,6-DNT	2,4,6-TNT			TOTAL
1	<0.25 - 17.2	<0.75 - 11.5	<0.10	<0.05 - 50.9	<0.10 - 146			≤1.70 - 158
2	<0.25	<0.75	<0.10 - 88.1	<0.05 - 113	<0.10 - 118			<1.35 - 249
3	<0.25	<0.75	<0.10	<0.05 - 58.0	<0.10 - 139			<1.35 - 198
4	<0.25 - 1.2	<0.75	<0.10 - 88.7	<0.05 - 109	<0.10 - 49.4			<1.35 - 160
5	<0.25 - 1.0	<0.75 - 66.3	<0.10	0.3 - 53.7	<0.10 - 95.5			≤1.60 - 140
6	<0.25	<0.75 - 2.0	<0.10	1.9 - 32.5	<0.10 - 4.8			≤4.90 - 38.5
7	<0.25	0.9 - 2.2	<0.10	4.5 - 17.5	<0.10 - 0.3			≤5.85 - 20.4
8	<0.25	<0.75 - 39.3	<0.10	<0.05 - 3.9	<0.10 - 0.2			<1.35 - 40.6
9	<0.25	<0.75 - 38.4	<0.10 - 6.0	1.1 - 3.4	<0.10 - 0.4			≤4.00 - 40.9
EFF	<0.25	<0.75	<0.10 - 104	<0.05 - 60.0	<0.10 - 29.6			<1.35 - 195
24 hr comp EFF	<0.25	<0.75	<0.10 - 111	<0.05 62.0	<0.10 - 187			<1.35 - 361

Comments

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WATER CHEMISTRY 5/17/77 ch. 7

TABLE C-4

VAAP WATER CHEMISTRY
RANGE OF VALUES

Water and Air Research, Inc.

PROJECT ANALYTICAL DATA SUMMARY

ARMY MEDICAL CORPS V
Project

PHASE II
Project Phase

VOLUNTEER ARMY AMMUNITION PLANT

SAMPLED MARCH 1-5, 1977

STATION NUMBER	Range of Values							
	NH_3-N	TKN	NO_3-N	Cl	T.Hard	SO_4		
1	0.94 - 1.47	1.57 - 2.11	7.31 - 16.9	13.5 - 21.2	118 - 212	132 - 198		
2	0.95 1.49	1.53 - 2.64	8.16 - 13.9	15.0 - 20.5	122 - 202	139 - 198		
3	1.00 - 1.24	1.69 - 1.99	7.49 - 18.9	14.5 - 18.0	132 - 192	144 - 177		
4	0.99 - 1.17	1.81 - 1.99	7.24 - 18.3	13.8 - 17.0	128 - 190	153 - 175		
5	0.84 - 1.09	1.52 - 1.72	7.28 - 18.1	14.0 - 16.5	125 - 188	153 - 290		
6	0.26 - 0.68	0.57 - 1.22	3.15 - 7.71	11.0 - 14.5	112 - 147	56.0 - 122		
7	0.12 - 0.26	0.48 - 0.96	2.55 - 4.11	9.0 - 10.8	98.0 - 117	61.0 - 77.0		
8	0.08 - 0.16	0.40 - 0.71	1.40 - 2.25	8.3 - 9.3	88.0 - 94.0	32.3 - 48.7		
9	0.04 - 0.13	0.23 - 0.52	0.63 - 1.55	7.0 - 8.5	78.0 88.0	12.5 - 27.3		
EFF	1.02 - 1.57	1.63 - 2.25	7.25 - 21.8	14.0 - 24.0	118 - 230	146 - 193		
	mgN/l	mgN/l	mgN/l	mgCl/l	mg/l as $CaCO_3$	$mgSO_4$/l		

Comments

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APPENDIX D

PERIPHYTON METHODS

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METHODOLOGY FOR PERIPHYTON COLLECTION

Within the study area periphyton samples were collected on natural and artificial substrates. Forms colonizing natural substrates were collected at the end of February, while those on exposed glass slides were not collected until the end of March. For this reason, the data are not comparable except in a general qualitative sense.

Three samples representative of natural substrates were obtained at each site and preserved in 5 percent formalin. These were oxidized with hydrogen peroxide and potassium dichromate to remove organic matter. The treated sample was dried on an 18 x 18 coverslip, mounted in Hyrax (refractive index 1.7), and examined under oil immersion (1000X). For qualitative comparisons, a minimum of 500 diatom valves were identified per sample. Raw-count data were transferred to a coding sheet. This information was key punched prior to computer analysis. Several routines were utilized including culling the data to eliminate species which occurred only rarely. The collection of periphyton on glass slides is considered to give more reliable station-to-station comparisons than natural substrates because of influence by substrate specificity. Standard 1 x 3 inch microscope glass slides were placed in periphyton samplers (Periphytomer II)™ one inch below the water surface at nine stations located in Waconda Bay, and three stations located in the reference bay (Figure 3). Periphytometers were placed in suspension units as shown in Figure D-1. Samplers were placed according to recommended procedures outlined in Standard Methods for the Examination of Water and Wastewater 14th Edition, (APHA, 1975), and Biological Field and Laboratory Methods for Measuring the Quality of Surface Water and Effluents, (Weber, 1973).

Periphytometers were anchored in a northerly direction utilizing the suspension unit designed for this study. A total of 8 racks (64 slides) were placed in the suspension units at each station. As part of a supplemental statistical study to measure variability between replicate slides and to determine the best practical method for processing periphyton slides, additional 6-rack (48 slides) suspension units were placed at Stations 1, 3, 8, 9, 11 and 12. Anchorline adjustments were made for anticipated lake level fluctuations of 2 feet or less. All slides were collected over a 2-day period from March 31 - April 1, 1977, following an incubation period of 30 days. Sampling devices were recovered at all stations except 9 and 10, where periphytometer units had been overturned exposing slides to the air. However, at station 9, the statistical 6-rack unit of slides was still intact and was used as a substitute for the vandalized 8-rack units.

At the end of the 30-day incubation period, slides from both the 8-rack and 6-rack units were recovered utilizing the following collection procedures. End slides (Positions 1 and 8) from the 8-rack units were removed, dried, and placed into labeled glass bottles. Slides for biomass, chlorophyll a, diatom, filamentous algae, and vital stain counts were collected from slide positions 2 - 7 using the random number generator of a Texas Instruments SR-51A

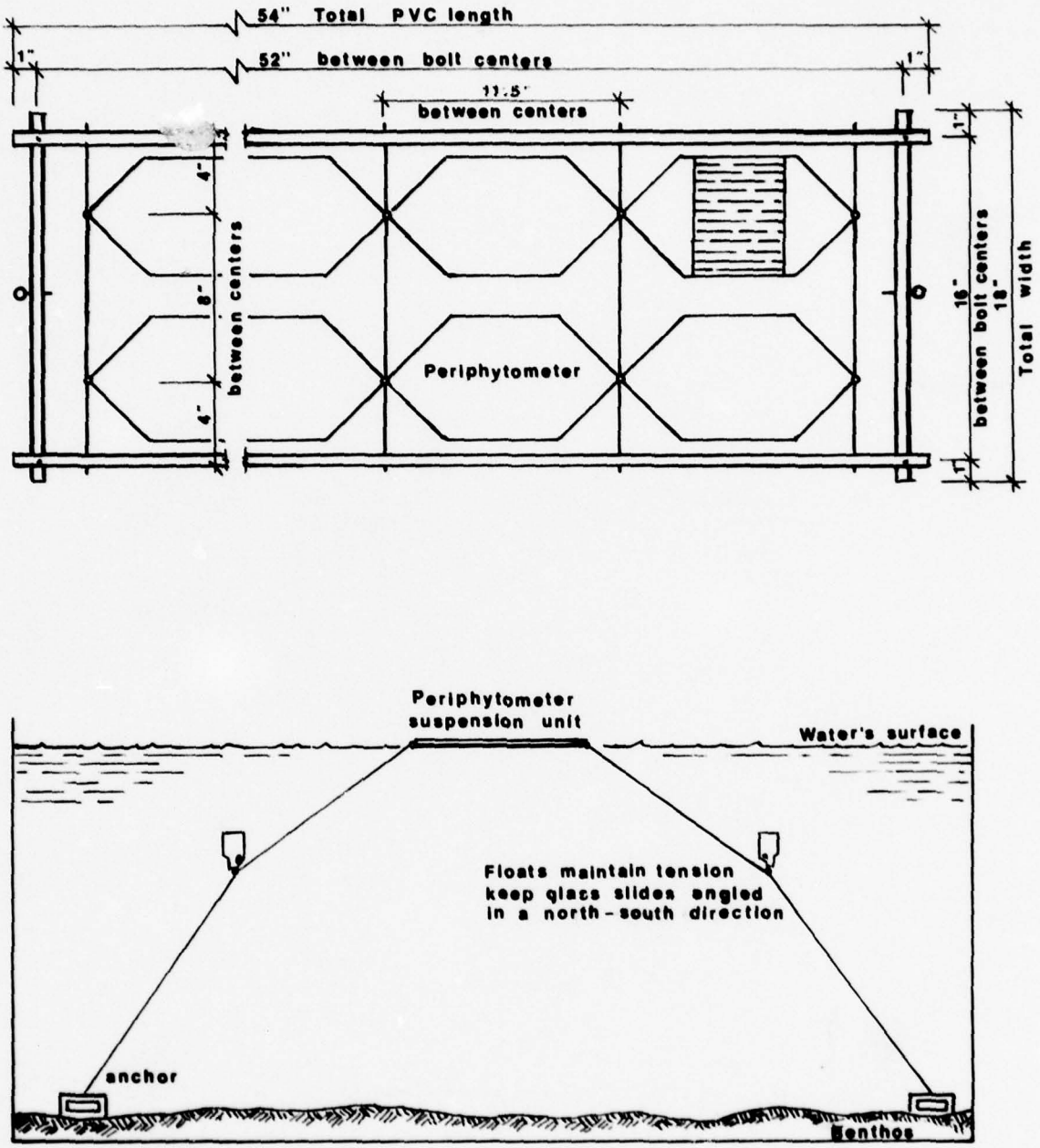

FIGURE D-1. PERIPHYTOMETER SUSPENSION UNIT

calculator. The priority for selecting slides from each rack was:

2 end slides	- Air-dried
2 slides	- Biomass determination
2 slides	- Chlorophyll *a*
1 slide	- Diatom counts
1 slide	- Filamentous algae or vital stain counts
8 slides	- Total

For the 8-rack periphyton suspension units the minimum numbers of slides for analyses were:

Analyses	Minimum Number of Replicates per Station
Chlorophyll *a*	16
Biomass	16
Diatom counts	5
Filamentous algae	2
Vital stain counts	3
Total	42

Slides from the 6-rack suspension units were all air-dried for diatom counts.

Chlorophyll a Determination

Except for Station 10, a total of 16 replicate periphyton slides were collected at each station for chlorophyll *a* analysis. While in the field, periphyton slides were placed in 50 ml of a 90 percent acetone (v/v), 10 percent of a saturated $MgCO_3$ solution and immediately stored in the dark on dry ice. Prior to analysis, chlorophyll was extracted for 24 hours in the dark at 4°C. To facilitate extraction, slides were scraped and the acetone suspension ground 30 seconds at 500 rpm in a Potter-type tissue homogenizer.

Following extraction chlorophyll *a*, corrected for phaeophytin, was determined fluorometrically after the methods of Yentsch and Menzel (1963), Holm-Hansen, *et al.* (1965), Lorenzen (1967), and Moss (1968), using a Turner Design Model 10 fluorometer. Fluorometric determination of chlorophyll depends on red fluorescence emitted by the chlorophyll *a* molecule when excited by ultraviolet light and is 100 times more sensitive than spectrophotometric analysis. The method is limited to chlorophyll *a* only; chlorophyll *b* and *c* cannot be determined.

The chlorophyll *a* reference solution was a purified spinach chlorophyll standard (Product No. C5753, Sigma Chemicals, St. Louis, MO) calibrated by spectrophotometric chlorophyll analysis.

Acidification of chlorophyll *a* converts it quantitatively to phaeophytin. Reading the fluorescence before and after adding one drop of 1N HCl to the sample cuvette allows calculation of an acid factor related to the interference.

Periphytic chlorophyll a was calculated as follows:

$$\text{Chlorophyll } a \text{ } (mg/m^2) = \frac{(F)(r)(Ca)(\text{ml extract})}{(r-1)[\text{substrate area (mm)} \ 10^{-3}]}$$

where: Ca = fluorometer reading before-fluorometer reading after acidification

$$r = \frac{\text{standard before acidification}}{\text{standard after acidification}}$$

$$F = \left[\frac{Ca}{\text{fluorometer reading}}\right] \left[\frac{\text{dilution ration fluorometer}}{\text{dilution ratio spectrophotometer}}\right]$$

Organic Biomass.

Sixteen replicate biomass slides were collected from each station. In the laboratory each slide was rehydrated for 15 minutes, accumulated material scraped from the slide into a graduated cylinder, and then resuspended in a total volume of 50 ml distilled water. An aliquot of the suspension was filtered on a tared, fired-glass filter (Gelman, GFA), the ash-free dry weight determined (APHA, 1975) and converted to grams of organic matter per square meter.

Diatom Cell Densities.

At least five replicate slides per station were examined for diatom community structure and cell density estimates. Periphyton growth was scraped from glass slides into tall labeled beakers using razor blades and a rubber policeman. Samples were oxidized with 20 ml of 50 percent hydrogen peroxide and approximately 50 mg of potassium dichromate. The solution was cooled, allowed to settle for 24 hours, decanted, and brought to a volume of 50 ml. Preliminary examination of oxidized material from Stations 6 - 12 indicated the samples were too concentrated to count accurately. Therefore, 4- and 8-fold dilution was necessary to permit observations of 10 - 15 organisms per microscope field. In contrast, samples at Stations 1 - 5 were too sparse and had to be concentrated by a factor of 5 to permit accurate counting.

Permanent slides were prepared by pipetting 0.4 ml of the "cleaned" material onto an 18 x 18 coverslip (324 mm^2) and allowing the sample to dry at 65°C (150°F) on a laboratory hot plate. The dried coverslip was placed on a standard microscope slide containing one drop of Hyrax mounting medium (refractive index 1.7) and the slide gently heated to drive off the toluene solvent. When cooled, the permanent slide was labeled with station number, date, location, and dilution factor. Under an oil immersion lens (Zeiss microscope, 1000X) diatoms were identified and enumerated to the species level, where possible, utilizing the following standard taxonomic references: Hustedt, 1930, 1962; Schmidt, et al., 1974-1959; Huber-Pestalozzi, and F. Hustedt, 1949; and Patrick and Reimer, 1966, 1975. Voucher diatom slides were sent to Dr. Charles Reimer, Academy of Natural Sciences of Philadelphia, for taxonomic verification.

Cell densities were estimated by performing field counts at 6-slide coordinates randomly selected on the coverslip. A total of 10 microscope fields were examined at each of the 6 selected coordinates for a total of 60 fields. At 1000X, each microscope field represented an area of 0.0182 mm^2

with a total area examined of 1.092 mm^2 (i.e. 0.0182 mm^2 x 60 fields). Therefore, cell densities were determined as follows:

$$\text{Cells/mm}^2 = \text{Diatom Counts} \times \frac{\text{Total Area of Coverslip (324 mm}^2\text{)}}{\text{Total Area Examined (1.092 mm2)}} \times \frac{\text{Original Volume of Periphyton Suspension (50 ml)}}{\text{Volume of Sample dried on coverslip (0.4 ml)}} \times \text{Dilution Factor} \Big/ \text{Original Surface Area of Slide (3,871 mm}^2\text{)}$$

Filamentous Algae. Two replicate periphyton slides per station were collected from artificial substrate units located in Waconda Bay and the reference bay. Slides were mechanically scraped with razor blades and preserved in 5 percent formalin. Clumped periphyton material was dispersed by blending samples in a Waring blender. Algal filaments were identified using a 50 ml plankton sedimentation chamber and a Zeiss invertoscope D. Identifications were carried to species level, where possible, utilizing the following standard references: Drouet (1968); Prescott (1962); and Desikachary (1956).

Vital Stain Counts. Recent studies have shown that natural and artificial substrate periphyton populations contain significant numbers of diatom frustules that are either empty or have less-than-robust chloroplasts (Pryfogle and Lowe, 1976). Standard diatom cleaning procedures using ammonium persulfate, acid or hydrogen peroxide oxidation do not distinguish between diatoms having metabolically active chloroplasts and those having empty frustules. Under certain conditions an investigator may wish to determine the metabolic state of diatoms in response to a pollution source. The following experimental vital stain procedure was conducted at 11 stations located in Waconda Bay and the reference bay using tetrazolium violet. Tetrazolium salts have been used to determine the dehydrogenase activities of bacterial populations in activated sludge and to a limited extent in the analysis of plankton populations (Armitage, 1977). The enzymatic reduction of the tetrazolium salt (yellow) to triphenyl formazin precipitate (violet stain) visually tags diatom cells with active cytochrome systems. As a result, cell counts can be qualitatively differentiated into one of three metabolic categories:

Metabolic Activity	Cell Description
Viable or active	Violet precipitate present throughout cell, or concentrated in mitochondria.
Senescent	Chlorophyll present, no violet precipitate formed.
Dead or fossil forms	No violet precipitate or chlorophyll present in frustule.

Three replicate periphyton slides per station were removed from the samplers and placed in glass bottles (75 ml) containing lake water collected at the site. These samples were then placed in the dark and 1 ml of a 0.2 percent (w/w) solution of paraiodotetrazolium was added to the sample bottle.

The slides were incubated in the light for a period of 12 hours after which they were preserved with 3 percent formalin. Vital stain counts were made utilizing a 50 ml plankton sedimentation chamber with a Zeiss inverted microscope equipped with an oil immersion 1000X lens. Diatom populations were differentiated into metabolically active, senescent, or dead (fossil).

APPENDIX E

STATISTICAL METHODS

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STATISTICAL METHODS

I. Introduction

The statistical analyses of the artificial substrate periphyton data considered the following conditions:

- comparison of variability in density and diversity estimates between fields on a single slide with variability between aliquots.
- comparison of variability in density and diversity estimates between fields and aliquots with variability between slides.
- comparison of variability in density and diversity estimates when one slide is used with that when a composite of five slides is used.
- comparison of the eleven sampled stations with respect to diversity and density.
- at one station a procedure for determining dominant species to a specified degree of precision was determined.

Shannon's measure of diversity

$$\hat{H} = -\sum_{i=1}^{S} \hat{p}_i \ln \hat{p}_i,$$

was used where $\hat{p}_i$ is the proportion of the i^{th} species in the sample ($\hat{p}_i = \frac{n_i}{N}$), s is the number of observed species, and we use natural logarithms. Basharin (1959) has shown that $\hat{H}$ is a biased estimate of the population diversity

$$H = -\sum_{i=1}^{S} p_i \ln p_i$$

where p_i is the true proportion of species i in the population, and S is the true number of species present in the population. The bias of $\hat{H}$ is negative, implying that $\hat{H}$ tends to underestimate H. Basharin shows that

$$\text{Bias} = -\frac{s-1}{2N}$$

where N is the total number of organisms observed. Thus a bias-adjusted estimate of H

$$\hat{H}_B = \hat{H} + \frac{s-1}{2N}$$

can be obtained. Basharin also derives the standard error of $\hat{H}_B$ showing that

$$S.E.(\hat{H}_B) = \frac{1}{N}\left[\sum_{i=1}^{S} p_i \ln^2 p_i - H^2\right]$$

which can be estimated by

$$\hat{S.E.}(\hat{H}_B) = \frac{1}{N}\left[\sum_{i=1}^{s} \hat{p}_i \ln^2 \hat{p}_i - \hat{H}_B^2\right]$$

This allows one to measure the reliability of the diversity estimate and to compare the station diversities statistically.

Turning to density, the logarithmic transformation

$$L_i = \ln(N_i + 1)$$

was used to transform N_i, the total number of organisms per observational unit (usually a group of fields) to a variable L_i which more nearly satisfies the assumptions necessary for an analysis of variance. Specifically, the variable L_i will often have a distribution which is approximately normal when N_i is skewed to the right. This transformed density measure can now be used to make statistical comparisons of the various sampling procedures, and of the stations.

II. Comparison of Diversity and Density Variability Between Fields With That Between Aliquots

A. Description of Experiment

One slide was selected from each of four stations, 3, 8, 11, and 12. Ten aliquots were taken from each slide, and 120 fields or 500 organisms, whichever came first (with a 60 field minimum), were counted on the microscope slide made from each aliquot. Counts were made in units of 10 fields. This experiment enables one to compare the variability between fields to that between aliquots at each station.

B. Diversity

As shown in Section VIII-A, one may analyze the bias-adjusted diversities using a nested analysis of variance model. Table E-1 presents the results of the analysis for the four stations.

C. Density

The same nested analysis of variance model which was used for diversity was used to analyze L, the logarithmic transform of density. The results are shown in Table E-2.

D. Conclusion

Whether the replicates consist of groups of fields from one microscope slide, or of fields from different aliquots, one can pool the results when estimating diversity and density. There is no evidence that the aliquot variability and the field within aliquot variability differ.

TABLE E-1

COMPARISON OF DIVERSITY VARIABILITY BETWEEN ALIQUOTS
TO THAT BETWEEN FIELDS FOR 4 STATIONS

Station	Source	DF	SS	MS	F
3	Aliquot	9	3.4132	.3792	1.00
	Fields(Aliquot)	110	41.826	.3802	
8	Aliquot	9	0.1648	.01831	0.46
	Fields(Aliquot)	60	2.4041	.04007	
11	Aliquot	9	0.1731	.01923	0.86
	Fields(Aliquot)	50	1.1240	.02248	
12	Aliquot	9	0.3569	.03966	1.06
	Fields(Aliquot)	70	2.6068	.03724	

None of the F values are significant at even the .10 level. There is no evidence that aliquoting introduces variability into the estimate of diversity.

TABLE E-2

COMPARISON OF DENSITY VARIABILITY BETWEEN ALIQUOTS
TO THAT BETWEEN FIELDS FOR 4 STATIONS

Station	Source	DF	SS	MS	F
3	Aliquot	9	1.9445	.2161	.81
	Fields(Aliquot)	110	29.3589	.2669	
8	Aliquot	9	1.1033	.1226	.70
	Fields(Aliquot)	60	10.5679	.1761	
11	Aliquot	9	1.4106	.1567	1.01
	Fields(Aliquot)	50	7.7954	.1559	
12	Aliquot	9	1.7829	.1981	.98
	Fields(Aliquot)	70	14.1604	.2023	

As with diversity, there is no evidence to suggest that aliquoting contributes variability to the estimate of density.

III. Comparison of Diversity and Density Variability Between Slides To That Within Slides

A. Experiment

Nine slides were selected from each of the stations 3, 8, 11, and 12, and a count was obtained for 60 fields (10 fields at each of 6 random locations) on a single microscope slide prepared from each experimental slide. Thus the variability between slides can be determined, and then compared to that within slides.

B. Diversity

The variance estimate of diversity between slides is based on the 60 field counts of the single microscope slide; while the within slide variability is estimated from the 10 field counts from the previous experiment. Since the same stations are sampled, one would expect approximately six times more organisms in 60 fields than in 10, which theoretically increases the diversity variance by a factor of six. An appropriate test statistic to compare these variance components is therefore

$$F = \frac{6(\text{Variance Between Slides})}{(\text{Variance Within Slides})}$$

The results of this test are summarized in Table E-3.

The five percent level of F is about two in each case. It appears that the between slide variability is larger than the within slide variability at least at stations 8 and 11. That is, there is evidence that the diversity variance between slides may be slightly larger than that within slides.

C. Density

The assumption that the logarithmic transform L of density is approximately normal plays a key role in the comparison of density variability between and within slides. Using standard results for the lognormal distribution, one can find an estimate of within slide variance to be

$$S^2_W = e^{2\bar{y}_L + S^2_L}(e^{S^2_L} - 1)$$

where $\bar{y}_L$ is the mean of the log transform L and S^2_L is the variance of L. The same formula can be used with the between slide data to calculate S^2_B, the estimate of between slide variability. Then one can test that the between slide variability exceeds the within slide variability by computing

$$F = \frac{S^2_B}{6S^2_W}$$

TABLE E-3

COMPARISON OF DIVERSITY VARIABILITY
BETWEEN SLIDES TO THAT WITHIN SLIDES

Station	Source	DF	SS	MS	F
3	Between Slides	8	00.6010	0.07152	1.13
	Within Slides	119	45.2396	0.3802	
8	Between Slides	8	0.1385	0.01731	2.79
	Within Slides	69	2.5688	0.03723	
11	Between Slides	8	0.08544	0.01068	2.91
	Within Slides	59	1.2971	0.02199	
12	Between Slides	8	0.07891	0.009864	1.58
	Within Slides	79	2.9638	0.03751	

where the factor of 6 is present because 6 times more fields are involved in each density count for the between slide calculations. The results of the analysis are given in Table E-4. Note that this F test is only approximate, since the distributions are log-normal rather than normal.

D. Conclusion

There appears to be slightly more variability between slides than within slides for both diversity and density. The implication is that one should obtain replication by sampling several slides rather than aliquoting a single slide. This will protect against the possibility that the sampled station has more variability between than within slides, as some of the experimental ones did. The number of slides which must be sampled will be considered in Section VI.

IV. Comparison of Diversity and Density Variability When One Slide is Used With That When Five Slides Are Composited

A. Experiment

Five slides were composited and one microscope slide formed, on which 60 fields were counted. Five replicates of this experiment were performed at each of four stations, 3, 8, 11 and 12.

B. Diversity

In order to determine whether the compositing reduces variability, one can compare the composited variance to the single slide variance from the previous experiment (see III-A). The results are summarized in Table E-5.

C. Density

The same comparison can now be made on the logarithmic transforms of the densities (see Table E-6).

D. Conclusion

The compositing of five slides does not seem to reduce the variability of either diversity or density estimates. Since more replication (and thus better variance estimates) can be obtained by analyzing the slides separately, the single slide method is probably preferable to compositing slides.

V. Station Comparisons

A. Experiment

Since the previous results indicated that a replicated, single slide experiment at each station was preferred, this experiment was performed at seven other stations, five replicates at each station.

B. Diversity

The stations, their bias-adjusted diversity, and the standard error (see Basharin, 1959) are given in Table E-7.

TABLE E-4

COMPARISON OF DENSITY VARIABILITY BETWEEN SLIDES TO THAT WITHIN SLIDES

Station	Source	DF	Variance	F
3	Between Slides	8	250.05	2.99
	Within Slides	119	13.95	
8	Between Slides	8	17,489.1	3.42
	Within Slides	69	853.06	
11	Between Slides	8	9,293.4	1.10
	Within Slides	59	1,409.7	
12	Between Slides	8	12,394.2	2.35
	Within Slides	79	880.88	

There is ample statistical evidence to conclude that the density variability between slides exceeds that within slides at stations 3, 8, and 12.

TABLE E-5

COMPARISON OF COMPOSITED DIVERSITY VARIANCE
TO SINGLE SLIDE VARIANCE

Station	Source	DF	Variance	F
3	Single	8	.07152	1.21
	Composite	4	.05920	
8	Single	8	.01731	1.46
	Composite	4	.01185	
11	Single	8	.01068	3.29
	Composite	4	.00325	
12	Single	8	.009864	0.84
	Composite	4	.01170	

Since the F value for significance at the 0.10 level is F = 3.94, no evidence that composite diversity variance exceeds single slide variance is found.

TABLE E-6

COMPARISON OF COMPOSITED DENSITY VARIANCE
TO SINGLE SLIDE VARIANCE

Station	Source	DF	Variance	F
3	Single	8	.1129	15.6
	Composite	4	.007224	
8	Single	8	.1236	2.85
	Composite	4	.04330	
11	Single	8	.03815	0.38
	Composite	4	.1017	
12	Single	8	.04572	0.62
	Composite	4	.07419	

No consistent pattern in these variance comparisons is found. There seems to be significantly more variance in the single slide experiment than the composite slide experiment at Station 3, but somewhat less at Station 11.

TABLE E-8

POPULATION DENSITIES FOR ALL STATIONS

Station	Density (number/mm^2)
11	37,293
8	27,803
9	22,462
12	19,698
7	18,936
6	14,911
5	750
4	174
1	144
3	85
2	28

Thus the conclusion is that the station densities are aligned as follows:

11 > 9, 12, 7, 6, 5, 4, 1, 3, 2

8 > 6, 5, 4, 1, 3, 2

9, 12, 7, 6 > 5 > 4 > 1, 3 > 2

TABLE E-7

DIVERSITIES FOR ALL STATIONS

Station	Diversity	Standard Error
6	0.716	0.035
7	1.189	0.033
5	1.608	0.043
8	2.045	0.025
9	2.175	0.032
4	2.440	0.074
11	2.557	0.020
1	2.573	0.074
12	2.790	0.019
2	2.918	0.089
3	2.929	0.061

An analysis of variance comparison was deemed inappropriate due to the probable inequality of the diversity variances. Thus the stations were compared in a pairwise fashion. The lines on the left of the station numbers reflect the results of comparing the diversities by using the two sample Z statistic, connecting stations which cannot be declared significantly different. The formula for Z is

$$Z = \frac{(\text{Station i Diversity} - \text{Station j Diversity})}{\sqrt{(\text{Standard Error i})^2 + (\text{Standard Error j})^2}}$$

Since Basharin (1959) shows that Shannon's diversity estimate is asymptotically normal, this statistic should approximate a standard normal random variable when the true population diversities are the same, and it does not necessitate equal variances. When the value of Z is large (negative or positive), the implication is that the true station diversities differ. A conservative .01 significance level, Z = 2.58, was used since so many comparisons were made. Table E-7 shows that the stations' true diversities are arranged as follows:

$$6 < 7 < 5 < 8 < 9 < 4, 11, 1 < 12, 2, 3$$

C. Density

The logarithmic density transforms were subjected to a standard analysis of variance. The station comparison yielded a highly significant F value of 319.81, leaving no doubt that the station densities differ. The transformed densities were then subjected to Duncan's (1975) multiple comparison procedure to determine which stations differ. The estimated densities (untransformed) are shown in Table E-8, with the solid lines on the left of the station numbers connecting those stations which one cannot conclude as different at the .05 significance level.

VI. Sample Size Determination

Perhaps the most crucial decision which must be made in planning an experiment is the sample size, or number of replicates to be processed. One can use information gleaned from previous experiments to plan more carefully.

In Section III it was concluded that replication of slides was more desirable than replication of aliquots from a single slide. Hence, it was decided that the effect on a single slide consist of counting ten adjacent fields at each of six randomly selected locations (60 total fields). The question then becomes: how many slides should be sampled?

A. Diversity

Since diversity is analyzed on an untransformed scale, it is easier to work in absolute rather than percentage differences. The following equation (Steel and Torrie 1960) is for the absolute difference between two stations' diversities which can be detected with 90 percent confidence for n replicate slides:

$$D = 2(1.645)\sigma_1 \sqrt{\frac{2}{n}} = 4.653\sigma_1 / \sqrt{n}$$

TABLE E-8

POPULATION DENSITIES FOR ALL STATIONS

Station	Density (number/mm^2)
11	37,293
8	27,803
9	22,462
12	19,698
7	18,936
6	14,911
5	750
4	174
1	144
3	85
2	28

Thus the conclusion is that the station densities are aligned as follows:

11 > 9, 12, 7, 6, 5, 4, 1, 3, 2

8 > 6, 5, 4, 1, 3, 2

9, 12, 7, 6 > 5 > 4 > 1, 3 > 2

where σ_1 is the standard deviation of the diversity based on single slide estimates. This assumes that the stations being compared have the same standard deviation σ_1. The probability of falsely concluding a difference exists is set at $\alpha = .10$.

A plot of D versus n for the four experimental stations is shown in Figure E-1. Note that for all but Station 3, where the density of organisms was very low, one can detect a diversity difference of slightly less than 0.3 with 90% confidence using five replicate slides.

B. Density

Since the statistical analysis of density is performed on a logarithmic scale, the differences one can detect between station densities will be expressed in percentages. The absolute percentage difference which can be detected with 90% confidence, with $\alpha = .10$, is given by

$$P = 100\{\exp[4.653\sigma_1/\sqrt{n}\,] - 1\}$$

where σ_1 is the standard deviation of the transformed density for single slide estimates. A plot of p versus n for the four experimental stations is shown in Figure E-2. Note that one must have at least five replicate slides before he can be 90% sure of detecting density differences of 100%. It is obvious that density comparisons require more replication than do diversity comparisons to achieve similar reliability.

C. Conclusions

In order to determine the necessary sample size for estimating and comparing station diversities or station densities, a preliminary estimate of the variance, σ_1^2, must be obtained. One procedure is to count approximately 60 fields on a single slide from the station of interest, and then to estimate σ_1^2 by the following.

Diversity:

$$\vartheta_1^2 = \frac{1}{N}\left[\sum_{i=1}^{s} \hat{p}_i \ln^2 \hat{p}_i - \hat{H}_B^2\right] \quad \text{(See Section I for notation definitions)}$$

Then the number of slides necessary to detect a difference, D, in diversities with 90 percent confidence for two stations having approximately the same standard deviation is given by

$$n = 21.65\,\vartheta_1^2/D^2$$

If the stations of interest have very different variances, compute the average variance to use in the formula for n,

Density: The computation of an estimated variance for the density is more difficult. Perhaps the best method is to sample 3 slides from each station, count 60 fields on each slide, and then calculate the sample variance, s_1^2, for the 3 log-transformed density observations. Then the total number of slides which need to be sampled in order to detect a

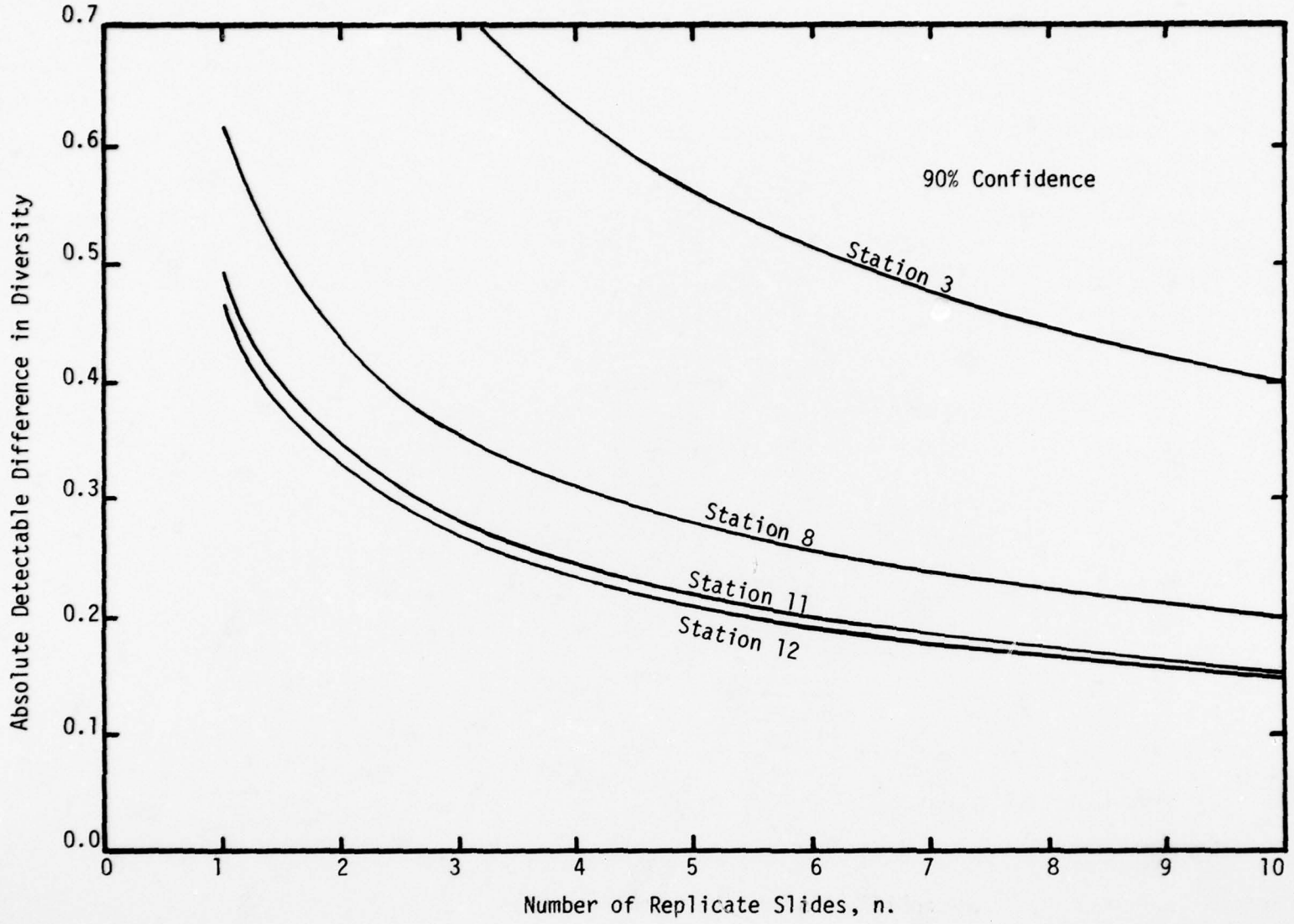

FIGURE E-1. SAMPLE SIZE TO MEET DIVERSITY CRITERIA

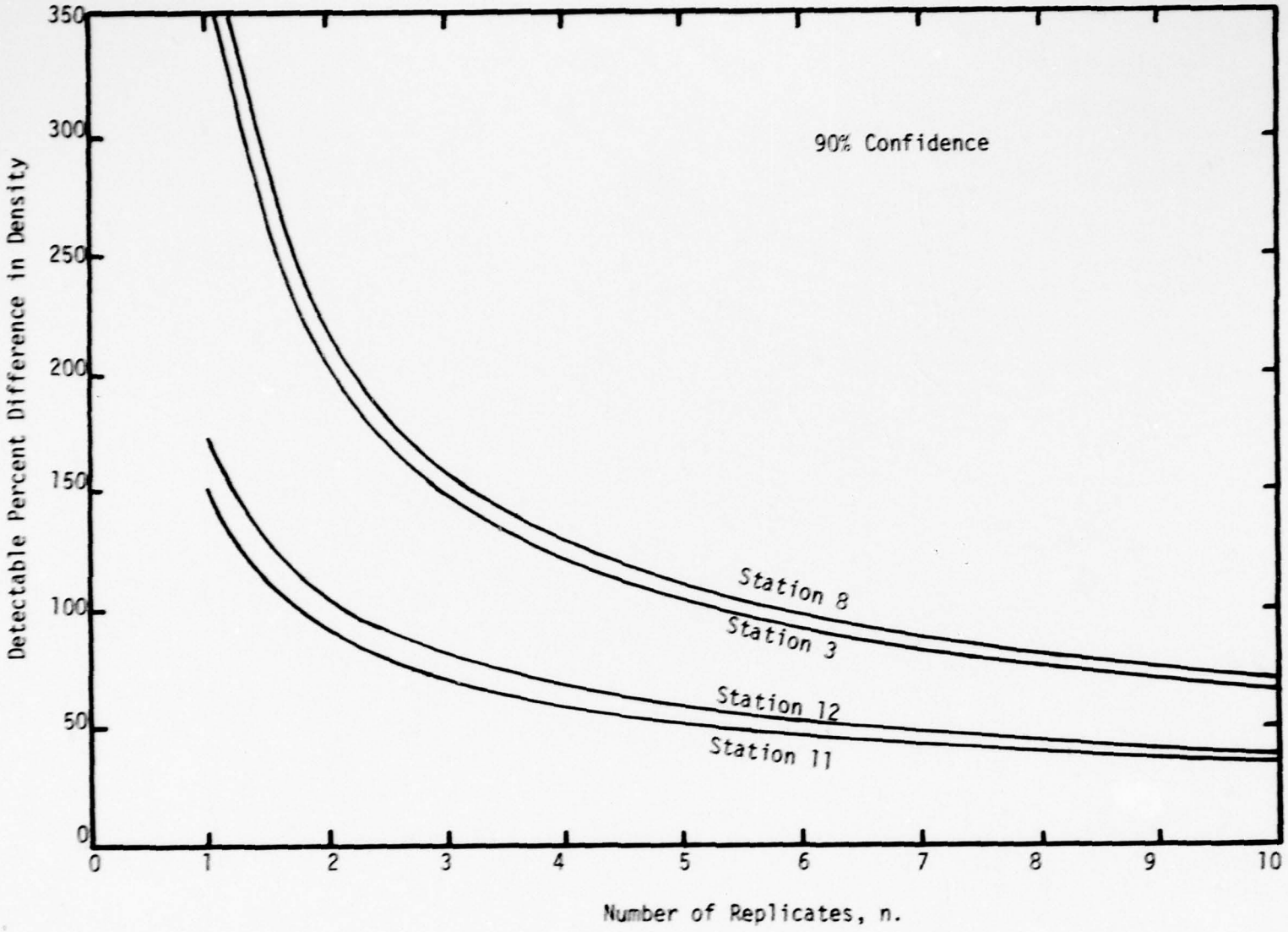

FIGURE E-2. SAMPLE SIZE TO MEET DENSITY CRITERIA

TABLE E-9

DOMINANT SPECIES FOR STATION 8, PERIPHYTON,
ARTIFICIAL SUBSTRATE

Organism		Slide No. 1	2	3	4	5	6	7	8	9	9-Slide Total
Achnanthes minutissima	No.	56	35	12	27	27	40	27	13	21	258
	%	8.0	7.9	5.1	7.5	8.1	12.5	7.2	6.1	7.4	7.9
Cymbella prostrate v. auerswaldii	No.	23	19	6	12	16	5	10	10	6	107
	%	3.3	4.3	2.6	3.3	4.8	1.6	2.7	4.7	2.1	3.3
Diatoma tenue v. elongatum	No.	284	179	120	131	150	95	145	83	130	1317
	%	40.8	40.1	51.1	36.2	44.9	29.7	38.8	39.0	45.9	40.4
Fragilaria vaucheriae	No.	138	77	40	100	54	64	51	36	47	607
	%	19.8	17.3	17.0	27.6	16.2	20.0	13.6	16.9	16.6	18.6
Melosira varians	No.	33	23	8	8	7	15	13	10	8	125
	%	4.7	5.2	3.4	2.2	2.1	4.7	3.5	4.7	2.8	3.8
Synedra rumpens	No.	81	56	26	47	38	44	75	26	29	422
	%	11.6	12.6	11.1	13.0	11.4	13.8	20.1	12.2	10.3	12.9
All other species	No.	82	57	23	37	42	57	53	35	42	428
	%	11.8	12.8	9.8	10.2	12.6	17.8	14.2	16.4	14.8	13.1
Total	No.	697	446	235	362	334	320	374	213	283	3264

percentage difference P between two stations is given by

$$n = 21.65\ s_1^2/[\ln(1+P/100)]^2$$

Again, s_1^2 may be obtained by averaging the various station variances if preliminary samples are obtained for several stations.

VII. Determination of Dominant Species

An analysis of dominant species was conducted using 9 replicate slides at Station 8. The basis for dominant species selection was that each species make up at least 3% of the pooled sample at the station. Six species satisfied this criteria at Station 8. The data are shown in Table E-9.

The first question to be answered was: Can one pool all nine replicates and treat each species composition estimate as a binomial estimate. For example, the total count of species 1 in all replicates was 258, which is 7.9% of the total count of 3264. If one can use binomial theory, the 95% confidence interval for species 1 percent composition is

$$7.90 \pm 2 \times \sqrt{\frac{(7.9)(100 - 7.9)}{3264}}$$

or

$$7.90 \pm 0.94$$

Thus, one would estimate with 95% confidence that between 6.96 and 8.84 percent of the population consists of species 1.

A χ^2 contingency table test was conducted to determine whether there is significant difference between replicates. The table consisted of 7 rows corresponding to the 6 dominant species and all others combined and 9 columns corresponding to the 9 replicate slides. The χ^2 statistic of 110.8 is significant at the .0001 level when compared to the χ^2 distribution with 48 degrees of freedom. Thus, it is concluded that a significant difference exists between the replicate estimates of percent composition for the 6 species. Since the replicates are all taken from the same population (station), the percent composition of each replicate estimates the true composition at the station. The fact that the χ^2 test shows a significant difference in replicate percentages warns that the pooling and use of binomial theory is inadvisable.

Using the theory given in Section VIII-B, we can obtain a sample estimate of variability without assuming the binomial distribution applies. Both the binomial and sample estimates are shown in Table E-10.

It is obvious that if one were to use the binomial formula for variance, the result would be an underestimate. Thus, in determining the sample size necessary to estimate the percent composition for a species with a specified accuracy, the binomial formula will underestimate the necessary sample size.

Several methods can be employed to more realistically determine the sample size necessary. For example, suppose one wanted to estimate the percent composition attributable to species 3, Diatoma tenue v. elongatum (from Table E-9), the most dominant, to within 2% with 95% confidence. Although the binomial bound is already less than 2% (1.72%) with 3,264

TABLE E-10

VARIANCE AND BOUND BASED ON BINOMIAL THEORY AND ACTUAL SAMPLING RESULTS

Species	Estimate	Variance		95% Confidence Bound	
		Binomial	Sample	Binomial	Sample
1	7.90	.0727	.1203	0.94	1.21
2	3.28	.0317	.0401	0.62	0.70
3	40.35	.2407	1.0856	1.72	3.64
4	18.60	.1514	.7033	1.36	2.94
5	3.83	.0368	.0509	0.67	0.79
6	12.93	.1126	.3205	1.17	1.93

organisms counted, the more realistic sample estimate is 3.64%. The number of organisms necessary to reduce this bound to 2% is found by solving:

$$n = \frac{4\ (\text{Estimated Variance})}{(\text{Bound})^2} \times 10^4$$

$$= \frac{4(1.0856)}{(2)^2} \times 10^4 = 10{,}856$$

This works out to about 21 more replicate slides at the present rate of approximately 363 organisms per slide (60 fields).

The main disadvantage of the above method is that one must have an estimated sample variance before n can be calculated. Thus, several replicates (at least three would be advisable) must be analyzed before the necessary sample size can be estimated. A cruder alternative is available when preliminary sampling cannot be conducted. First, one can estimate the sample size using the binomial formula, then multiply by a "safety factor" to adjust for replicate variability. The appropriate factor is the ratio of the true variance to the sample variance. In our example, Table E-10, the factor ranges from about 1.5 for the species at the lower end of the dominant scale to about 4 for the species with high percent composition.

For example, if one were to use the binomial variance to estimate the sample size necessary to estimate the species 3 percent composition to within 2%, with 95% confidence, one would get:

$$n = \frac{4\ (\text{Binomial Variance})}{(\text{Bound})^2} \times 10^4$$

$$= \frac{4(.2407)}{(2)^2} \times 10^4 = 2407$$

Using the safety factor of 4, since species 3 is at the upper end of the dominant percentages, one finds that about 9600 organisms should be counted. This is not too far from the estimate of 10,856 obtained using the sample results.

In summary, if slide replicates are to be used for estimating percent compositions, the variability of the estimates may be considerably more than is expected assuming a binomial distribution. More realistic estimates of variability can be calculated from the actual sample results, and these in turn can be used to estimate sample sizes necessary for a specified accuracy of estimation. If the binomial distribution is used to determine sample size, a safety factor should be used to adjust for replicate variability. To be safe, this factor should be at least 2 for species at lower percentages (5-10%), and 3 to 4 for species above 10%.

VIII. Detailed Procedures

A. Analysis of variance for Shannon's Diversity

Suppose one is estimating a station's true diversity by selecting a single slide, preparing n_A aliquots (and thus n_A microscope slides), and then counting n_c sets of 10 fields each on each slide (see section II of this report for the analysis of such an experiment). A statistical model for such an experiment would be:

$$H_{jk} = H + A_j + \varepsilon_{jk}$$

where H_{jk} is the Shannon diversity estimate for the k^{th} group of 10 fields in aliquot j

H is the true Shannon diversity,

A_j is a random effect associated with aliquot j

ε_{jk} is a random effect associated with the k^{th} group of 10 fields on aliquot j.

Assume:
$$E(A_j) = 0,\ Var(A_j) = \sigma_A^2/N_j$$
$$E(\varepsilon_{jk}) = 0,\ Var(E_{jk}) = \sigma_\varepsilon^2/N_{jk}$$
$$Cov\ (A_j, \varepsilon_{jk}) = 0$$

where N_{jk} = number of organisms in k^{th} group of 10 fields in aliquot j

$N_j = \sum_{k=j}^{n_c} N_{jk}$ = number of organisms in aliquot j

Thus
$$E(H_{jk}) = H$$
$$Var\ (H_{jk}) = \sigma_A^2/N_j + \sigma_\varepsilon^2/N_{jk}$$

Basharin (1959) shows that,

$$Var\ (H_{jk}) = [\sum_{i=1}^{S} Pi \ln^2 Pi - H^2]\ /N_{jk}$$

so that this model is in agreement to the order of N_{jk}.

Now one can apply the usual analysis of variance methods to this nested model for H_{jk}. First calculate

$$\bar{H}_j = \sum_{k=1}^{n_C} H_{jk}/n_C \qquad j = 1, 2, \ldots, n_A$$

$$\bar{H} = \sum_{j=1}^{n_A} \sum_{k=1}^{n_C} H_{jk}/n_A n_C$$

In terms of the model,

$$\bar{H}_j = H + A_j + \sum_{k=1}^{n_C} \varepsilon_{jk}/n_C$$

$$\bar{H} = H + \sum_{j=1}^{n_A} A_j/n_A + \sum_j \sum_k \varepsilon_{jk}/n_A n_C$$

so that

$$\mathrm{Var}(\bar{H}_j) = \frac{\sigma_A^2}{N_j} + \frac{\sigma_\varepsilon^2}{n_c^2} \sum_{k=1}^{n_C} \frac{1}{N_{jk}}$$

$$= \frac{\sigma_A^2}{N_j} + \frac{\sigma_\varepsilon^2}{n_c^2 \underline{N}_j}$$

where $\underline{N}_j = \left(\sum_{k=1}^{n_C} \frac{1}{N_{jk}} \right)^{-1}$

$$\mathrm{Var}(\bar{H}) \approx \frac{\sigma_A^2}{n_A^2} \sum_{j=1}^{n_A} \frac{1}{N_j} + \frac{\sigma_\varepsilon^2}{n_A^2 n_c^2} \sum_{j=1}^{n_A} \sum_{k=1}^{n_C} \frac{1}{N_{jk}}$$

$$\approx \frac{\sigma_A^2}{n_A^2 \underline{\underline{N}}} + \frac{\sigma_\varepsilon^2}{n_A^2 n_c^2 \underline{\underline{N}}}$$

where $\underline{\underline{N}} = \left(\sum_{j=1} \frac{1}{N_j} \right)^{-1}$, $\underline{\underline{\underline{N}}} = \left(\sum_j \sum_k \frac{1}{N_{jk}} \right)^{-1}$

Define

$$SSA = n_c \sum_{j=1}^{n_A} (\bar{H}_j - \bar{H})^2$$

$$= n_c \sum_{j=1}^{n_A} [(\bar{H}_j - H) - (\bar{H} - H)]^2$$

$$= n_c \sum_{j=1}^{n_A} (\bar{H}_j - H)^2 - n_c n_A (\bar{H} - H)^2$$

Thus

$$E\{SSA\} = \frac{n_c \sigma_A^2}{\underline{N}} + \frac{\sigma_\varepsilon^2}{n_c \underline{\underline{N}}}$$

$$- \frac{n_c \sigma_A^2}{n_A \underline{\underline{N}}} - \frac{\sigma_\varepsilon^2}{n_A n_c \underline{\underline{N}}}$$

$$= (n_A - 1)\left[\frac{n_c \sigma_A^2}{\underline{N}} + \frac{\sigma_\varepsilon^2}{\underline{\underline{N}}}\right]$$

$$E\{MSA\} = E\left\{\frac{1}{n_A - 1} SSA\right\} = \frac{n_c \sigma_A^2}{n_A \underline{N}} + \frac{\sigma_\varepsilon^2}{\underline{\underline{N}} n_A n_c}$$

Secondly,

$$SSC = \sum_{j=1}^{n_A} \sum_{k=1}^{n_c} (H_{jk} - \bar{H}_j)^2$$

$$= \sum_{j=1}^{n_A} \sum_{k=1}^{n_c} [(H_{jk} - H) - (\bar{H}_j - H)]^2$$

$$= \sum_{j=1}^{n_A} \sum_{k=1}^{n_c} (H_{jk} - H)^2 - n_c \sum_{j=1}^{n_A} (\bar{H}_j - H)^2$$

$$E(SSC) = \frac{(n_c - 1)\sigma_\varepsilon^2}{n_c \underline{\underline{N}}}$$

$$E(MSC) = E\left\{\frac{1}{(n_c - 1)n_A} SSC\right\} = \frac{\sigma_\varepsilon^2}{n_A n_c \underline{\underline{N}}}$$

If one forms the approximate* F ratio,

$$F = \frac{MSA}{MSC}$$

he gets a test of the hypothesis Ho: $\sigma_A^2 = 0$, or that the aliquoting contributes no extra variability (beyond that of the sets of fields within aliquots) to the diversity estimate. Large values of F would imply that $\sigma_A^2 > 0$, or that the aliquoting does contribute additional variability. These tests are conducted in section II. B.

Note that this same reasoning could be extended to additional hierarchies. For example, one could similarly analyze an experiment involving stations, slides within stations, and fields within slides within stations. In this way one can determine which variance components seem to contribute most to the variability of the diversity estimate. These tests were conducted in sections II to IV of this report.

B. Procedure for Estimation of Percentage of Specific Organism

Assume r replicate slides, with $n_1, n_2, \ldots, n_r$ organisms identified on each slide. Let $y_1, y_2, \ldots, y_r$ represent the number of a particular species in each replicate. If p is the true fraction of the population which is the species of interest, then one may estimate p by the r fractions

$$\hat{p}_1 = \frac{y_1}{n_1}, \hat{p}_2 = \frac{y_2}{n_2}, \ldots, \hat{p}_r = \frac{y_r}{n_r}$$

Central limit theory implies that these sample fractions will be approximately normally distributed, with mean p. If the binomial assumption is made, the variance of $\hat{p}_i$ is $\frac{p(1-p)}{n_i}$. In the periphyton sampling experiment, there is evidence that this value underestimates the actual variance, hence it is proposed that the assumption

$$\text{Variance } (\hat{p}_i) = \frac{\sigma^2}{n_i} \quad \text{be used}$$

where σ^2 is an unknown constant, exceeding p(1-p). One can estimate σ^2 by the following sample statistic:

$$s^2 = \frac{\sum_{i=1}^{r} n_i(\hat{p}_i - \hat{p})^2}{r - 1}$$

where $\hat{p}$ is the pooled estimate of p, i.e.

$$\hat{p} = \frac{\sum_{i=1}^{r} y_i}{\sum_{i=1}^{r} n_i} = \frac{\sum_{i=1}^{r} y_i}{N}$$

*Basharin (1959) proves that the diversity estimate is asympotically normal, which lends credence to the assumption that this ratio possesses an F distribution.

Then use $\hat{p}$ to estimate p, but instead of estimating the variance of $\hat{p}$ by $\frac{\hat{p}(1-\hat{p})}{N}$, use

$$\text{Var}(\hat{p}) \simeq \frac{s^2}{N}$$

To estimate a percentage composition to within a specified bound B and with a confidence level of C percent, first find the z value from the normal tables corresponding to C, i.e. z = 2 for C = 95 percent, z = 1.65 for C = 90 percent, etc. Then solve

$$n = \frac{z^2 s^2}{B^2} \times 10^4$$

where the 10^4 factor is used of B is given as a percent, but eliminated if B is given as a decimal fraction.

IX. Biologic Interpretation of Results

The statistical analysis has shown that a significant source of variability in determining either diversity or density on artificial substrate periphyton samples is the difference between fields on an individual slide. In the procedure utilized here, the slides were prepared by placing a 0.4 ml aliquot on an 18 x 18 mm coverslip. A random number procedure was used to determine the location on the coverslip to conduct the actual count. Ten adjacent fields were counted at each selected coordinate, i.e. if 60 total fields were counted, six locations on the coverslip were utilized.

The data for the separate 10 field units shows a considerable variation in total numbers. Locations near the edge of the coverslip tend to have much lower organism density than locations near the center. The reason for this is that when the 0.4 ml aliquot dries, it does so from the edge to the center. This has the effect of concentrating organisms at the center of the coverslip. Hence, there is a very uneven distribution of diatoms that is particularly apparent when a random number procedure is utilized to determine the counting position on the coverslip.

Given the variation described above, it is possible that the significance of aliquot to aliquot or slide to slide variation may have been masked. Hence, if the variation due to drying of the aliquot on the coverslip could be reduced then the aliquot to aliquot and slide to slide variation could be assessed in a more precise manner. A re-examination of these variables would be appropriate.

An obvious needed improvement in processing technique is the development of a procedure that does not concentrate organisms near the coverslip center. Lacking this, it is important that a randomized procedure be used to select coverslip locations which counteracts the natural tendency to count near the center where most of the organisms are concentrated.

APPENDIX F

MACROINVERTEBRATE METHODS

LIST OF FIGURES

METHODOLOGY FOR MACROINVERTEBRATE COLLECTION

Aquatic macroinvertebrates were collected from natural and artificial substrates in March, 1977, at 12 stations. Natural substrates were sampled with a petite Ponar dredge. Hester-Dendy artificial substrates were suspended approximately 1.5 - 3.0 feet below the surface. Fifteen replicates of the natural substrate, and fourteen units of artificial substrates, were collected at each station to minimize natural variability.

To decrease variability a special unit was fabricated for Hester-Dendy plate suspension (Figure F-1). This sampling device was designed and built by Mr. B.C. Pruitt of Water and Air Research, Inc. The unit consists of PVC pipe molded into a circle with a circumference of approximately 2-1/2 feet. Fourteen 5 plate units are attached to the suspension frame by wing nuts. Floats are attached to a line of predetermined length. The entire apparatus is anchored at selected station locations. For purpose of this survey, units were suspended 18 inches below the surface.

In the field, dredge samples of the natural substrate were washed in a bucket sieve (U.S. Standard No. 30 mesh) and bottled. Rose Bengal dye was then added to facilitate laboratory sorting. Samples were preserved in 10 percent formalin. Natural substrate samples were rewashed in the laboratory and picked in a white enamel pan partially filled with water. After sorting, organisms were placed in vials containing 95 percent ethanol. Chironomid larvae were mounted in polyvinyl-lactophenol for microscopic identifications.

Samples from all stations were analyzed and identifications were made to the lowest practical taxonomic level. Fifteen natural substrate replicates were processed for stations 1, 5, and 11, and five were processed for the remaining nine stations. Five of the 14 artificial substrate replicates (whole units) were processed for all twelve stations. The community structure indices which were computed include the Shannon-Weaver diversity index and the Pinkham-Pearson index of biotic similarity.

Organism Loss From Processing

A problem encountered with the quantification of benthic macroinvertebrates was the loss of organisms during laboratory processing. This occurs when benthic samples are rinsed in a 30-mesh sieve to remove formalin, dye, and the finer sediments. Therefore, we saved the rinse water and examined it for macroinvertebrates for the five replicates at Station 1 in order to estimate losses from this technique. This experiment was unnecessary for artificial substrates since they were not rinsed in a sieve.

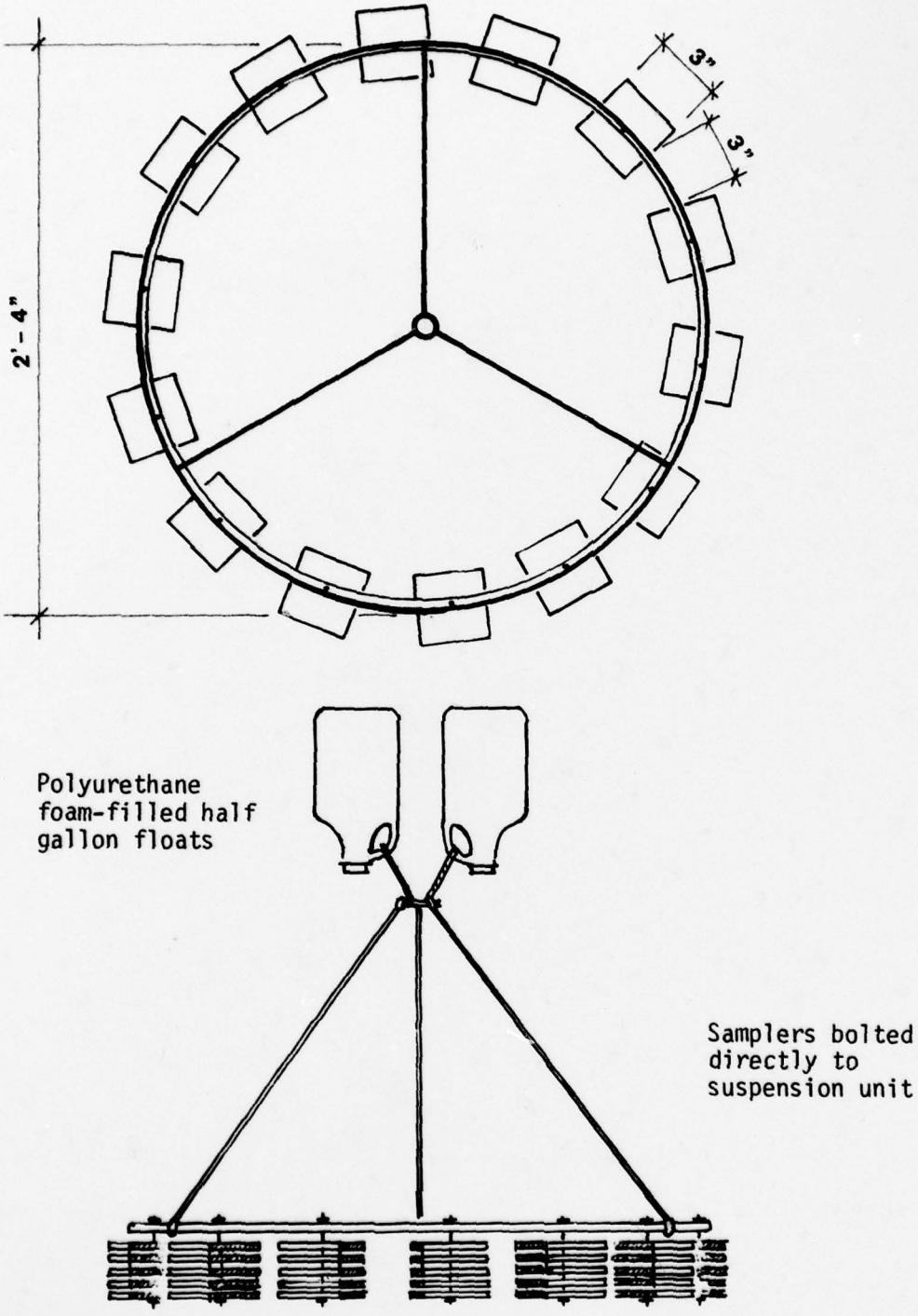

FIGURE F-1. HESTER DENDY ARTIFICIAL SUBSTRATE SUSPENSION UNIT.

The table below, with the five replicates pooled, enumerates the results:

Taxa	Number of Organisms Retained By:		
	30-mesh Sieve	Rinse Pan	
Procladius sp.	88	3	
Tanypus neopunctipennis	7	3	
Oligochaeta	1.5	0.5	
Nematoda	1	0	
Unidentified Coleopteran	1	0	
Totals	98.5 (93.8%)	6.5 (6.2%)	Σ=105

A similar experiment was conducted on a leaf pack sample. Of an estimated 400-600 organisms within the sample, approximately 100-200 passed through the sieve. Almost all those lost were chironomids and we feel that a 6.2 percent loss is acceptable. The loss is apparently only partially related to organism size when considering those with small diameters. Experience has shown that there is very little loss when samples contain moderate to large amounts of filamentous algae or sand. Apparently, sand plugs many of the sieve openings and filamentous algae entangle the organisms which results in higher sieve retention.

The sediment in the study area was composed almost entirely of clay and silt. This matrix was of a pudding-like consistency. It was typically orange or brown (oxidized) on top, becoming gray (reduced) underneath. The top orange layer, as observed from dredged benthic grabs, was about 1-2 cm at Stations 1 and 2. It was 2-4 cm, at Stations 3, 4 and 5, but reduced to a thin veneer at Station 6. The orange coloration was replaced by brown at Stations 7 through 11. It was a brownish-orange at Station 12, grading to medium brown below. Some gravel remains of an old dam were found at Station 9. Small amounts of shell were observed at Stations 6 through 11.

Detritus was present at all stations, but was abundant only at Station 6. Oil was also found only at Station 6 and formed a film on the water surface when sieving macroinvertebrate samples. A mat of filamentous algae covered the bottom at Stations 3 through 6 where it was relatively dense. However, it was very thin at Station 6. A slender spikerush (Eleocharis acicularis?) was found in small quantities on the benthos at Station 12.

Dead fish were present in the sediment at Stations 1 through 5. They were partially decomposed and almost unidentifiable but were probably gizzard shad and appeared most abundant at Stations 1 through 3. Burrows of the mayfly larvae Hexagenia sp. were abundant at Stations 7 through 9 while only a few were found at Stations 10 and 11.

An effort was made to sample benthic macroinvertebrates at comparable depths. This was possible at all stations except 8, 9, 10 and 12. Stations 8, 9, and 10 were 3-4 meters deep, and Station 12 was about 0.75 meters deep. The remaining stations were 1.3-2 meters deep.

APPENDIX G

COMPUTATIONAL METHODS

LIST OF FIGURES

COMPUTATIONAL METHODS*

Community Analysis

Introduction

Biotic components of water quality are generally quantified by one-dimensional diversity indices when single samples or stations are examined, or two-dimensional coefficients of biotic similarity when sample/sample, station/station, or species/species comparisons are undertaken.

Diversity indices are mathematical expressions that describe the distribution of individuals within the community. There are a number of diversity expressions in use. In general, maximum diversity exists if each individual belongs to a different species. An environmental parameter that influences community structure will also modify the diversity index. In cases where environmental stress may occur (such as competition among species, physiochemical limiting factors, or pollution), the community is reduced in the number of species present. Frequently, this reduction in the number of species is accompanied by an increase in the number of individuals of the remaining species, especially in the case of organic pollution. Environmental stress, therefore, tends to reduce the magnitude of diversity indices. One-dimensional diversity indices include the Shannon-Weaver Species Diversity, Evenness, and Simpson's Index of Dominance.

Coefficients of biotic similarity quantify the taxonomic overlap between two samples or stations. Most of these coefficients assume values between 0 and 1, where a value of 0 indicates no species overlap, and a value of 1 implies identical species composition. Morisita's Index of Faunal Affinity and the Pinkham-Pearson Index of Biotic Similarity are measures of biotic similarity.

In this study, data processing subsequent to manual taxonomic identification/confirmation was executed through the IBM 370/OS system at the Northeast Regional Data Center of the State University System of Florida (NERDC). Diversity indices and coefficients of similarity were calculated by proprietary FORTRAN IV routines. The phenograms were generated through application of the NT-SYS Numerical Taxonomy System developed by Rohlf, Kishpaugh and Kirk at Stony Brook (1974).

Shannon-Weaver Species Diversity Index (H)

The Shannon Weaver Species Diversity Index, $\bar{H}_e$ (Odum, 1971) is defined as:

$$\bar{H}_e \equiv \sum_{i=1}^{t} \frac{n_i}{N} \ln \frac{n_i}{N}$$

*Procedures outlined and examples cited were part of a study conducted for the U.S. Army Medical Research and Development Command.

where n_i = total number of organisms present as species i

$N = \sum_{i-1}^{t} n_i$ = total number of organisms present in the sample

t - number of taxa present in the sample.

$\bar{H}_e$ ranges from a minimum of 0.0, occurring when all organisms belong to the same taxon (no diversity), to a maximum of ln N, occurring where each organism present belongs to a unique taxon (maximum diversity).

The Shannon-Weaver Index is commonly expressed to other logarithmic bases, especially base 2 and base 10, and is easily converted by the following expression:

$$\bar{H}_{base_x} = \frac{\bar{H}_e}{\ln x}$$

Pinkham-Pearson Index of Biotic Similarity (B)

The previously discussed index ($\bar{H}$) quantifies community structure with a sacrifice of taxonomic integrity important to paired comparisons between samples or stations. Such an index is incapable of distinguishing samples of similar gross community structure, but unlike taxonomic composition. That is, in computation, the i th species of one sample is not necessarily the same i th species of another sample.

This insensitivity to taxonomic overlap is surmounted by the Pinkham-Pearson Index of Biotic Similarity, B (Pinkham and Pearson, 1974) defined as:

$$B \equiv \frac{1}{t} \sum_{i=1}^{t} \frac{\text{Min } (n_{iA}, n_{iB})}{\text{Max } (n_{iA}, n_{iB})}$$

where t = number of taxa considered

n_{iA} = number of organisms of species i present at Station A

n_{iB} = number of organisms of species i present at Station B

Min (n_{iA}, n_{iB}) = the minimum value of the pair: n_{iA}, n_{iB}

Max (n_{iA}, n_{iB}) = the maximum value of the pair: n_{iA}, n_{iB}

Biotic similarity is defined only for a paired comparison between two samples or stations. If two samples are characterized by identical taxonomic overlap (all species occur in identical abundance), the calculated index assumes a value of 1.0 (maximum similarity). Two samples possessing no species in common share an index of 0.0 (minimum or no similarity). The number of species considered, t, may include only those species observed in either or both of

the two samples, or, if mutual absence is deemed important, may include species not necessarily present in either sample. If mutual absence is considered important, Min (0,0) ≡ 1 and Max (0,0) ≡ 1 in the computation of biotic similarity.

A biotic similarity index, B', between species may be defined on spatial and numerical occurrence by transposition of the axes in the preceding expression of station similarity:

$$B' \equiv \frac{1}{k} \sum_{j=1}^{k} \frac{\text{Min } (n_{j1}, n_{j2})}{\text{Max } (n_{j1}, n_{j2})}$$

where: k = number of samples or stations considered

n_{j1} = number of organisms of species 1 at Station j

n_{j2} = number of organisms of species 2 at Station j

Min (n_{j1}, n_{j2}) = the minimum value of the pair: n_{j1}, n_{j2}

Max (n_{j1}, n_{j2}) = the maximum value of the pair: n_{j1}, n_{j2}

This index likewise ranges from 0.0 (minimum similarity) to 1.0 (maximum similarity). B' may possess utility for grouping species according to environmental preference or pollution tolerance -- that is, it may delineate "indicator organisms".

Phenograms

The quantification of similarity between paired stations, samples, or species by any of the previously-defined coefficients of similarity generates a diagonal matrix containing PC unique elements, where PC is calculated from the expression (Pinkham and Pearson, 1974):

$$PC = \frac{S\ (S - 1)}{2}$$

where: PC = number of unique paired comparisons

S = number of stations, samples, or species being compared.

For a study comprising only 25 stations, a similarity matrix of 300 unique elements is produced. Evaluation and presentation of such a voluminous matrix is impractical without computer-aided analysis and graphic models.

Algorithms for clustering similarity matrices into two-dimensional, hierarchic relationships have been developed by numerical taxonomists (Sokal and Sneath, 1963). A technique frequently invoked by ecologists and generally regarded as introducing the least distortion into similarity relationships is the sequential, agglomerative, hierarchic, nonoverlapping clustering method (SAHN) using unweighted pair-groups with arithmetic averaging (UPGMA), described by Sokal and Sneath (1963). The product of this procedure is a branched diagram termed a phenogram (or dendrogram), illustrated in Figure G-1.

Figure G-1.

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Use Figure 10

This phenogram was generated by computer using the NT-SYS Numerical Taxonomy Package (Rohlf, Kishpaugh, and Kirk, 1974).

The horizontal scale or abscissa of the phenogram is graduated in the units of the similarity measure upon which the clustering was based -- in this case, the Pinkham-Pearson Biotic Similarity Index. Points of furcation (branching) between the horizontal stems, representing stations or groups of stations imply that the similarity between the two streams is at the coefficient value shown above the branch on the abscissa.

The magnitude of similarity between any two stations represented on the phenogram will, in general, differ from the corresponding magnitude given in the original similarity matrix. This arises as a consequence of the averaging necessary to recursively agglomerate the separate stations into a single, structured set containing all the stations.

The degree of distortion resulting from the cluster analysis may be quantified by the cophenetic correlation coefficient, r_{coph}, defined as the product moment correlation coefficient computed between the elements of the original similarity matrix and the corresponding indices implied by the phenogram (Sokal and Sneath, 1963). High values of r_{coph} ($r_{coph} > 0.8$ for fewer than 10 stations) indicate that the distortion introduced by the clustering procedure and depicted by the phenogram has not significantly masked the informational content of the original similarity matrix.

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